Synthesis of Non Protein Bound Amino Acids

THESIS SUBMITTED TO
THE ALIGARH MUSLIM UNIVERSITY, ALIGARH
FOR THE DEGREE OF
Doctor of Philosophy
IN CHEMISTRY

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M. Sc., M. Phil.
Dec. 1975

ABSTRACT

A B S T R A C T

A general method for the synthesis of non-protein bound amino acids from azlactones is described. Catalytic hydrogenation of azlactones in alcoholic ammonia using Raney nickel and palladium-charcoal (10% Pd) as catalysts generally gave high yields of N-benzoylamino acid amides although yields varied with the catalyst used. Reduction of 2-phenyl-4-phthalidene-5-oxazolone in this manner gave a product in which both the lactone and oxazolone rings underwent ammonolysis. Hydrolysis of the N-Benzoylamino acid amides, using a variety of conditions, produced either the required α-amino acids or their N-benzoyl-derivatives.

AZLACTONES

Azlactones used in this work were prepared by condensing carbonyl compounds with hippuric acid in presence of acetic anhydride and different base catalysts.

Sodium acetate was used in the synthesis of 2-phenyl-4-(1'-naphthylmethylene)-5-oxazolone (62.8%), 2-phenyl-4(o-methoxybenzal)-5-oxazolone (75%), 2-phenyl-4-(2';4'-diacetoxybenzal)-5-oxazolone (91.5%), 2-phenyl-4-phthalidene-5-oxazolone (60.7%), 2-phenyl-4-(p-dimethylaminobenzal)-5-oxazolone (69.2%), 2-phenyl-4-cyclopentylidene-5-oxazolone (33%), 2-phenyl-4-cyclohexylidene-5-oxazolone (82.6%) and 2-phenyl-4-(3'-methoxy-4'-acetoxybenzal)-5-oxazolone (75%).

Potassium carbonate was employed for the preparation of 2-phenyl-4-(o-acetoxybenzal)-5-oxazolone (71%), 2-phenyl-4-(m-nitrobenzal)-5-oxazolone (100%), 2-phenyl-4-(o-nitrocinnamylidene)-5-oxazolone (98%), and 2-phenyl-4-crotonylidene-5-oxazolone (40%).

Lead acetate was used in the synthesis of 2-phenyl-4-(1';3'-dimethyl-3'-aminobutylidene)-5-oxazolone (36.2%), 2-phenyl-4-(1';3'-dimethyl-2'-butenylidene)-5-oxazolone (34%), and 2-phenyl-4-(1';2';2'-trimethyl-propylidene)-5-oxazolone (42%).

Potassium bicarbonate was also used as a catalyst for the preparation of 2-phenyl-4-(3'-pyridylmethylene)-5-oxazolone (92.2%), 2-phenyl-4-piperonalmethylene-5-oxazolone (82%), and 2-phenyl-4-crotonylidene-5-oxazolone (40%).

N-BENZOYLAMINO ACID AMIDES

N-Benzoylamino acid amides obtained by Raney nickel catalysed hydrogenation of azlactones in alcoholic ammonia were: DL-N-Benzoyl-β-1-naphthylalanine amide (90%), DL-N-benzoyl- -o-methoxyphenylalanine amide (61%), DL-N-benzoyl-β-2; 4-dihydroxyphenylalanine amide (78%), DL-N-benzoyl-β-3-methoxy-4-hydroxyphenylalanine amide (80%), DL-N-benzoyl-β-amino-β-(o-benzenecarbonamido)alanine amide (59%), DL-N-benzoyl-β-p-dimethylaminophenylalanine amide (65%), DL-N-benzoyl-cyclopentylglycine amide (63%), DL-N-benzoylcyclohexylglycine amide (81%), DL-N-benzoyl-o-tyrosine amide (82%), DL-N-benzoylnorleucine

amide (62%), DL-N-benzoyl-δ;δ-dimethyl-δ-aminoisoleucine amide (55%), DL-N-benzoyl-δ;δ-dimethylisoleucine amide (58%), DL-N-benzoyl-β;γ-dimethylleucine amide (62.4%), and DL-N-benzoyl-β-piperonylalanine amide (82.5%).

N-Benzoylamino amides obtained by palladium-charcoal (10% Pd) catalysed hydrogenation of azlactones in alcoholic ammonia were: DL-N-benzoyl-β-1-naphthylalanine amide (95%), DL-N-benzoyl-β-o-methoxyphenylalanine amide (77.4%), DL-N-benzoyl-β-2;4-dihydroxyphenylalanine amide (78%), DL-N-benzoyl-β-3-methoxy-4-hydroxyphenylalanine amide (85%), DL-N-benzoyl-β-amino-β-(o-benzenecarbonamido) alanine amide (64.5%), DL-N-benzoyl-β-p-dimethylaminophenylalanine amide (71%), DL-N-benzoyl-cyclopentylglycine amide (67%), DL-N-benzoylcyclohexylglycine amide (86%), DL-N-benzoyl-o-tyrosine amide (86%), DL-N-benzoyl-β-m-aminophenylalanine amide (86%), DL-N-benzoyl-δ-o-aminophenylnorvaline amide (98%), DL-N-benzoylnorleucine amide (68%), DL-N-benzoyl-δ;δ-dimethyl-δ-amino-isoleucine amide (69%), DL-N-benzoyl-δ;δ-dimethylisoleucine amide (68%), DL-N-benzoyl-β;γ-dimethylleucine amide (65%), DL-N-benzoyl-β-3-pyridylalanine amide (95%), and DL-N-benzoyl-β-piperonylalanine amide (85%).

N-BENZOYLAMINO ACIDS

DL-N-Benzoylamino acids were synthesised by mild hydrolysis of the corresponding amides using following hydrolysing agents.

Hydrochloric acid (36%) was employed in the synthesis of DL-N-benzoyl-β-1-naphthylalanine (75%), DL-N-benzoyl-β-o-methoxyphenylalanine (98%), DL-N-benzoyl-β-3-methoxy-4-hydroxyphenylalanine (72%), DL-N-benzoyl-cyclohexylglycine (93%), DL-N-benzoyl-o-tyrosine (70.5%), DL-N-benzoyl-norleucine (75%), DL-N-benzoyl-δ;δ-dimethylisoleucine (72.8%), DL-N-benzoyl-β;γ-dimethylleucine (97%), and DL-N-benzoyl-β-piperonyl-alanine (74%).

Hydrochloric acid (10%) was used for the preparation of DL-N-benzoyl-β-amino-β-(o-benzenecarboxylic acid) alanine (68%), DL-N-benzoyl-β-p-dimethylaminophenylalanine (54%), DL-N-benzoyl-β-m-aminophenylalanine (60%), DL-N-benzoyl-δ-o-aminophenylnorvaline (96%), DL-N-benzoyl-δ;δ-dimethyl-δ-aminoisoleucine (74%), and DL-N-benzoyl-β-3-pyridylalanine(71%).

Sodium hydroxide solution (20%) was used for the preparation of DL-N-benzoyl 2; 4-dihydroxyphenylalanine (78%), and DL-N-benzoylcyclopentylglycine (78%).

AMINO ACIDS

Hydrolysis of N-benzoylamino acid amides under reflux gave the corresponding amino acids with different hydrolysing agents.

Hydrochloric acid (36%)was used in the synthesis of DL-β-o-methoxy-phenylalanine (56%), DL-β-amino-β-(o-benzenecarboxylic acid) alanine (60%), DL-cyclopentylglycine (63%), DL-cyclohexylglycine (55%), DL-o-tyrosine (25%), DL-norleucine (80%), DL-δ;δ-dimethylisoleucine (78%), and DL-β;γ-dimethylleucine (82%).

Hydrochloric acid (10%) was employed for the preparation of DL-β-p-dimethylaminophenylalanine (71.5%), DL-β-m-aminophenylalanine (79%), DL-δ-o-aminophenylnorvaline (74%) DL-δ;δ-dimethyl-δ-aminoisoleucine (62%), and DL-β-3-pyridylalanine (95%).

Hydriodic acid (sp.gr.1.7) in presence of red phosphorus was used for the preparation of DL-β-1-naphthylalanine (75%), DL-β-2;4-dihydroxyphenylalanine (71%), DL-β-3;4-dihydroxyphenylalanine (90%), DL-cyclohexylglycine (64%), DL-o-tyrosine (89%), and DL-norleucine (95%).

Barium hydroxide solution (15%) was used for the hydrolysis of N-benzcyl-β-piperonylalanine amide giving β-piperonylalanine (73.5%).

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This is to certify that the thesis entitled "Synthesis of Non-protein Bound Amino Acids" is the original work of the candidate and is suitable for submission.

(N.H. KHAN)

A C K N O W L E D G E M E N T

I take this opportunity to acknowledge my indebtedness to Dr Naseem Hasan Khan whose valuable advice and constant help led to the development of the work described in this thesis.

I also express my heartfelt gratitude to Dr W. Rahman, Professor and Head, Department of Chemistry for providing the research facilities.

Thanks are due to my colleagues for their cooperation and useful discussions.

I am also grateful to the University for the financial assistance.

MOHD ALI

C O N T E N T S

EXPERIMENTAL

I. SYNTHESIS OF AZLACTONES

HYDROGENATION OF AZLACTONES

II. PREPARATION OF N-BENZOYLAMINO ACID AMIDES:RANEY NICKEL CATALYSED REDUCTION

PREFACE

Organic acids in which one or more hydrogen atoms other than the carboxylic group are replaced by an amino substituent are called amino acids. α-Amino acids are those which have an amino group α-to the carboxylic function. With few exceptions (the imino carboxylic acids) all possess the general structure $H_2N.CHR-CO_2H$ where the side chain R may be of diverse composition and structure, i.e. R = CH_3-, $(CH_3)_3C$-, 3-$HOC_6H_4CH_2$- etc.

Some α-amino acids are commonly found as constituents of the proteins and these are referred to as the protein amino acids but in addition a number of other amino acids occur in nature in free and combined states with other organic molecules. For example these have been detected in the circulatory system, in body tissues, among the products of excretion, as intermediates in the metabolic processes, as components of antibiotics, as bacterial decomposition products, and in plants.

In the search of effective chemotherapeutic agents, various amino acid analogs have been synthesised in recent years. Some of these have been shown to possess antitumor properties and others inhibit the growth of certain bacteria and viruses. 3;4-Dihydroxyphenylalanine, 3-methoxytyrosine and α-methyl-3;4-dihydroxyphenylalanine are used in the treatment of Parkinsons disease and hypertension. Substituted phenylalanines and o-methyltyrosine nitrogen mustards and γ-(alkylthio) lysine have found uses in cancer and neoplastic disease therapy. 5-Hydroxytryptophan, N$^{\varepsilon}$-(α-lipoyl) lysine and cycloleucine have also shown promise in the treatment of mental disturbance,

liver disease, acne and leukemia. α-Amino acid derivatives are also the basic starting materials for the synthesis of peptides some of which are also essential for the regulation of various body functions.

Amino acids are also components of a number of antibiotics, and there is some interest in effective incorporation of other amino acids in such biological active molecules. Moreover, the growth of disease producing micro-organisms, which utilise amino acids in their diet can be inhibited by certain amino acids which act as antimetabolites. There is also possibility that unnatural amino acid may also directly inhibit the growth of the parasite through incorporation of amino acid analogs in the proteins of their hosts. If such is the case, such an approach may lead to an inhibition of cell growth by introducing suitable amino acid analogs into cancer tissue proteins. Keeping all these points in mind it was thought worthwhile to try to develop a new and more efficient method for the synthesis of amino acids which utilised easily accessible and locally available starting materials.

A number of procedures are described in the literature for the synthesis of the protein amino acids but the preparation of non-protein amino acids has not been investigated in any great detail. An attempt has now been made to develop a suitable route for the synthesis of a number of non protein amino acids using azlactones as intermediates in the synthesis.

In this method a suitable azlactone is prepared which is hydrolysed to an acylaminoacrylic acid and then reduced to an N-benzoylamino acid. It has been shown that azlactones can be directly converted to the N-benzoylamino

acid amides in high yields and with greater purity by catalytic reduction in the presence of Raney nickel and alcoholic ammonia at elevated hydrogen pressure and room temperature. The resulting amides can be converted directly to the required α-amino acids or their N-benzoyl derivatives. Azlactones derived from m-nitrobenzaldehyde, o-nitrocinnamaldehyde, and pyridyl-3-aldehyde. · failed to undergo this reduction with Raney nickel but could be converted by palladised charcoal. An important aspect of the use of palladium charcoal for this purpose was that higher yields could be obtained as compared to the use of Raney nickel.

The various methods which have been used for the synthesis of non-protein amino acids are summarized in the following pages.

I N T R O D U C T I O N

I N T R O D U C T I O N

SYNTHESIS OF NON-PROTEIN BOUND AMINO ACIDS

I. METHODS BASED ON THE USE OF AMINATION OF α-HALOGEN ACIDS

(a) Direct Amination with Ammonia

(i) Synthesis of Amino Acids from Carboxylic Acids

α-Bromination of a fatty acid followed by treatment of the α-bromo acid with excess of aqueous ammonia forms the corresponding α-amino acid[1,2]. The -bromo acids from C_6 to C_{12} are not soluble in aqueous ammonia, therefore, alcoholic ammonia is required. Stearine[3] was synthesised using this method.

$$CH_3(CH_2)_{15}\text{-}CH_2\text{-}CO_2H + Br_2 \longrightarrow CH_3(CH_2)_{15}\text{-}\underset{Br}{CH}\text{-}CO_2H$$

$$\xrightarrow{NH_3} (CH_3(CH_2)_{15}\text{-}\underset{NH_2}{CH}\text{-}CO_2H$$

In the preparation of β-aminoalanine, found in hydrolysates of the antibiotic Viomycin, α, β-dibromopropionic acid was used[4]. Buston and Bishop[5] described the synthesis of β-hydroxyleucine starting from β-isopropylacrylic acid which was formed by treating isobutyraldehyde with malonic acid.

$$(CH_3)_2CHCHO + CH_2(CO_2H)_2 \xrightarrow[\text{reflux}]{C_5H_5N} (CH_3)_2\,CHCH{=}CHCO_2H$$

$$\xrightarrow{\text{Bromine water}} (CH_3)_2\underset{OH}{CH}\underset{Br}{CH}\text{-}CH.CO_2H \xrightarrow{NH_3} (CH_3)_2\,\underset{OH}{CH}\underset{NH_2}{CH}\text{-}CH.CO_2H$$

Similarly other β-hydroxy analogs of norleucine[6], valine[7] and norvaline[8] have been synthesised. Volhard[9] prepared sarcosine by treatment of ethyl chloroacetate with excess aqueous methylamine in a sealed tube at 120-130°.

$$ClCH_2CO_2Et + CH_3NH_2 \xrightarrow{120\text{-}130^\circ} CH_3\overset{H}{N}CH_2CONHCH_3$$

$$\xrightarrow{Ba(OH)_2} CH_3\overset{H}{N}CH_2CO_2H.$$

A similar synthesis involving α-halo acids or their esters and methyl or dimethylamines have been reported[10-12]. α-Aminoadipic acid[13], and fluoroamino acid analogs[14] have also been prepared by the direct amination of the respective halo acids.

(ii) Synthesis of Amino Acids from Substituted Malonic Acids

Direct halogenation of malonic acids proceeds much more smoothly than that of the corresponding monocarboxylic acids. Therefore, advantage has been taken of substituted malonic acids in the synthesis of α-amino acids.

Fischer and Blumenthal[15] synthesised homoserine via malonic ester using phenoxyethylbromide as the starting material. This amino acid occurs free in nature in peas and other green plants.

$$C_6H_5OCH_2CH_2Br + CH_2(CO_2Et)_2 \xrightarrow{NaOEt} C_6H_5OCH_2CH_2CH(CO_2Et)_2$$

$$\xrightarrow[2.\ Br_2]{1.NaOH} C_6H_5OCH_2CH_2\underset{Br}{C}(CO_2H)_2 \xrightarrow[2.NH_3]{1.heat} C_6H_5OCH_2CH_2\underset{NH_2}{CH}CO_2H$$

$$\xrightarrow[2.\ Ag_2CO_3,H_2S]{1.\ Conc.HBr\ heat} \underset{OH}{CH_2}\underset{NH_2}{CH_2}CH.CO_2H$$

Homocystine[16], the homolog of cystine, β-and γ-phenylserines[17] have also been synthesised using this method.

(iii) Synthesis of Amino Acids from Substituted Acetoacetic Esters

Takisawa[17] synthesised norleucine and other amino acids in 36-75 percent yields with appropriate alkylacetoacetic esters.

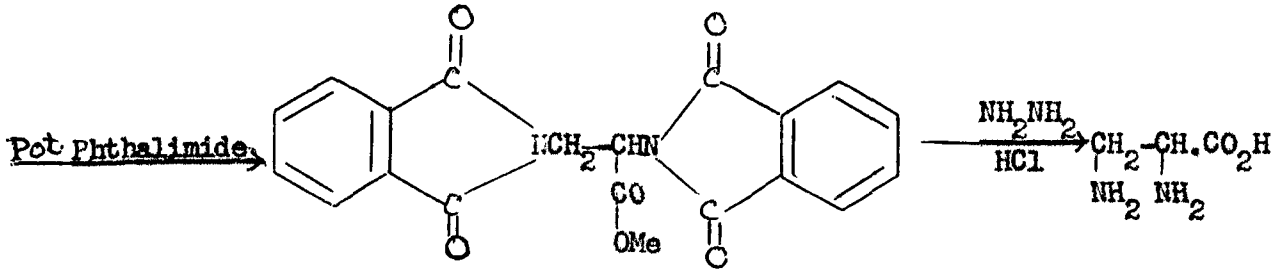
$$CH_3COCH(R).CO_2Et \xrightarrow{Br_2} CH_3COCBr(R).CO_2Et$$

$$\xrightarrow[2.\ H^+]{1.liq\ NH_3} R\text{-}\underset{NH_2}{CH}.CO_2H$$

(b) Synthesis of Amino Acids by Amination Using Gabriel Phthalimide Method

Shiv et al[18]. used potassium phthalimide in the synthesis of β-aminoalanine from methyl-α-bromo-β-(β-chloroethoxy) propionate.

$$ClCH_2CH_2OH + H_2C{=}CH.CO_2Me \xrightarrow[KBr,Br_2]{Hg(OAc)_2} ClCH_2CH_2OCH_2\underset{Br}{CH}.CO_2Me$$



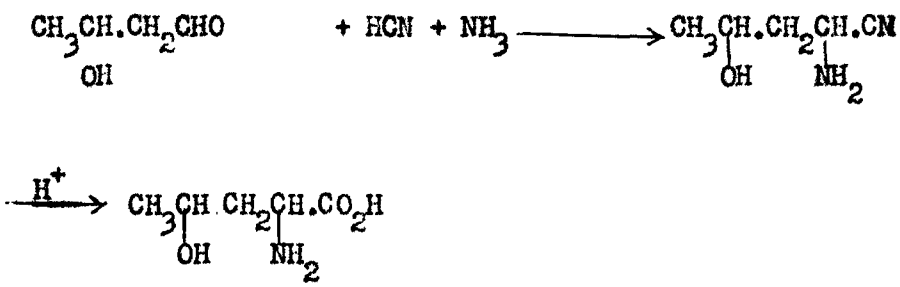
Using this method ornithine[19,20], an amino acid of shark liver, Tyrocidine and Gramincidin-S[1], α ; α'-diaminoadipic acid[21] and γ -hydroxyglutamic acid[22] have been synthesised.

II. METHODS BASED ON THE USE OF α-AMINONITRILE AND RELATED COMPOUNDS

(a) Synthesis of Amino Acids by Strecker Synthesis

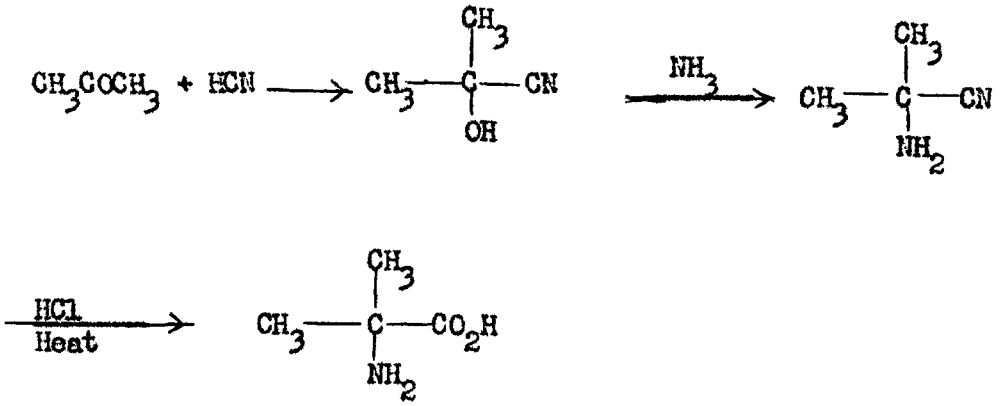
α-Aminonitrile was first used by Strecker[23] in 1850 in the synthesis of alanine. Therefore this method is often known as Strecker synthesis. He obtained the aminonitrile from aldehyde-ammonia. Later, other workers prepared α-aminonitriles from aldehyde cyanhydrins and also directly from aldehydes.

In 1902 Fischer and Leuchs[24] employed aldol with ammonia and hydrocyanic acid in a Strecker reaction affording γ-hydroxynorvaline in 30% yield.



Fischer and Fieldmann[25] employed this reaction for the synthesis of α-amino-$\beta;\gamma$-dihydroxybutyric acid. Goldfarb et al[26] prepared α-aminoheptylic acid, α-aminocaprylic and norleucine in about 50% yields. Other amino acids synthesised by this method are ornithine[27], indospicine[28], 4-pyrazolylglycines[29] and some sulfur containing amino acids[30].

Synthesis of α-alkylated amino acids, characterized by the lack of a hydrogen atom on α-carbon atom, involved the use of ketones in a Strecker reaction. Tiemann et al[31]. synthesised α-aminoisobutyric acid starting from acetone.

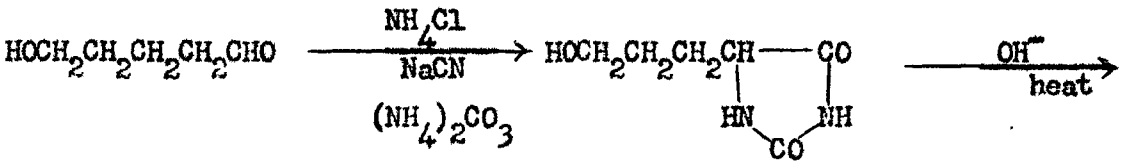


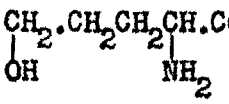
Gulewitsch and Wasmus[32] employed ammonium cyanide in place of hydrogen cyanide. Zelinsky and Standnikoff[33] used a mixture of pure potassium cyanide and ammonium chloride in the reaction with the appropriate ketone. $\alpha;\alpha'$-Diethylaminoacetic acid[31], α-methyl-α-phenylaminoacetic acid[31], isovaline[34], and other α-alkylated amino acids[35-39], o-carboranylalanine[40] and vinylglycine[40] have also been synthesised in the same manner.

Eschweider[41] synthesised sarcosine and N,N-dimethylglycine by treating methylamine and dimethylamine respectively with formaldehyde and hydrocyanic acid.

(b) Synthesis of Amino Acids by Bucherer Method

In this method a hydantoin is prepared by treating an aldehyde with a mixture of alkali cyanide and ammonium carbonate which is then hydrolysed to afford amino acid. . Gaudry[42] employed this method for the synthesis of γ-hydroxynorvaline starting from γ-hydroxybutyraldehyde.



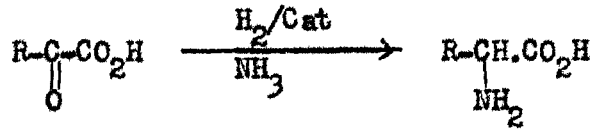


α-Aminoisobutyric acid[43], $\alpha;\alpha'$-diaminopimelic acid[44], α-methylthyroxine[45], S-benzyl-α-methylcysteine[46], heterocyclic ring substituted alanines[47] and α-methyl derivatives[48] of phenylalanine, glutamic acid and methionine have also been prepared using this method.

III. METHODS BASED ON THE USE OF KETO ACIDS AND THEIR ESTERS

(a) Synthesis of Amino Acids by Reduction of Keto Acids in the Presence of Ammonia

Knoop and Oesterlin[49] in 1925 for the first time carried out catalytic reduction of a number of α-keto acids to amino acids using platinum or palladium on carbon as catalyst in the presence of ammonia.



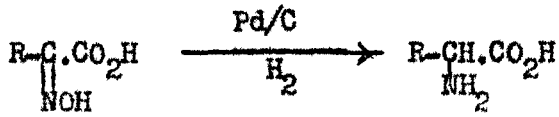
Using this method they synthesised α-and β-aminobutyric acids, α-amino-β-trimethylpropionic acid; α-, β-, γ-amino α-phenylacetic, β-phenylpropionic, γ-phenylbutyric and α-amino-phenylbutyric acids.

The main drawback of this method is that the desired keto acids are not always available.

(b) Synthesis of Amino Acids by Reduction of Oximes of Keto Acids and other Compounds

(i) By Reduction of Oximes of Keto Acids

Hartung et al.[50] obtained satisfactory yields of amino acids or their esters by reducing α-oximino acids or esters using palladium-charcoal catalyst.



α-Aminobutyric acid, norvaline, isoleucine, norleucine and O-methyltyrosine were the non-protein bound amino acids synthesised by this method.

Knoop and Landmann[49] synthesised tert-leucine starting from pinacolone. Synthesis of thienylalanine[51], α-aminoadipic acid[52] and α-aminopimelic acid[52] also involved more or less the same procedure.

(ii) By Reduction of Oximes of α-Bromo diester

α-Aminosuberic and α-aminosebacic acids[53] have been prepared in about 20% yields starting from α-bromoesters.

$$MeO_2C.\underset{\underset{Br}{|}}{CH}(CH_2)_5 \text{ (or 7)}. CO_2Me \xrightarrow{NaNO_2} MeO_2C.\underset{\underset{NOH}{||}}{C}-(CH_2)_5\text{(or 7)}.CO_2Me$$

$$\xrightarrow{Sn\text{-}HCl} HO_2C.\underset{\underset{NH_2}{|}}{CH}(CH_2)_5\text{(or 7)}. CO_2H$$

Oximes of α; α′-dibromosuberic or sebacic acids on reduction yielded the corresponding amino acids (loc.cit).

(iii) By Reduction of Oximes of Substituted Acetoacetic and other Esters

Hamlin et al[50]. prepared different homologs of amino acids by the palladium-catalysed reduction of oximes of substituted acetoacetic esters in the following manner:

$$CH_3COCHRCO_2Et \xrightarrow[H_2SO_4]{R'ONO} R\text{-}\underset{\underset{NOH}{||}}{C}.CO_2Et \xrightarrow[HOAc]{NaOH}$$

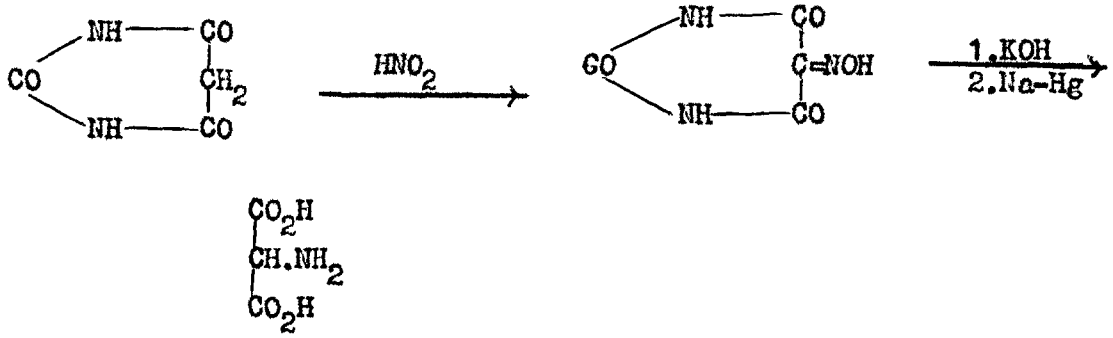
$$R\text{-}\underset{\underset{NOH}{||}}{C}.CO_2H \xrightarrow{2H} R\text{-}\underset{\underset{NH_2}{|}}{CH}.CO_2H$$

3-Hydroxyglutamic acid[54] and β; γ -dihydroxyglutamic acid[55] have been synthesised by catalytic reduction of oximes of diacetone and diethyl-acetone dicarboxylates respectively. Other amino acids obtained by this method are β -phenylglutamic acid[56], 4-pyridylalanine[57], 2-hydroxytryptophan[58], β -diazophenylalanine[59] and 4-quinilylalanine[57].

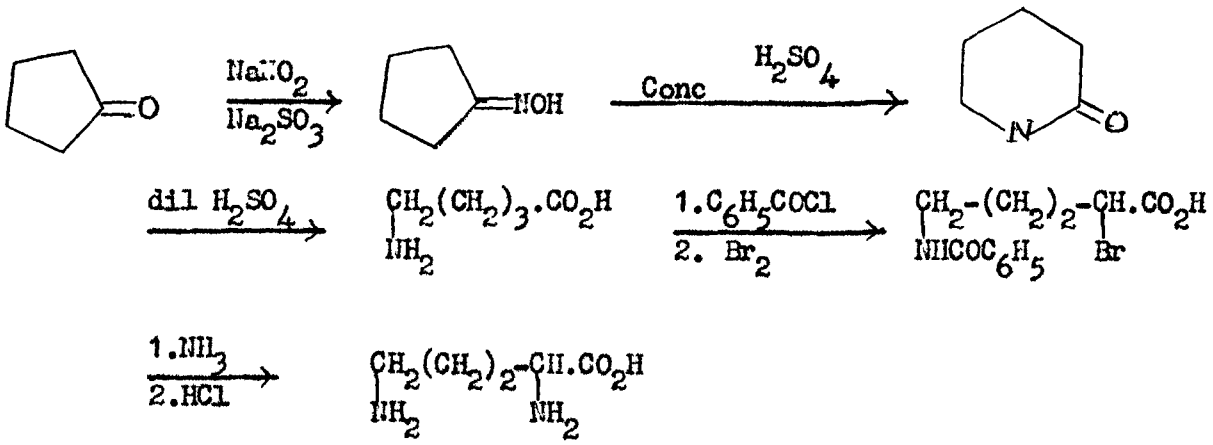
Synthesis of α-amino acids with unsaturated side chains[62] e.g., allylglycine, methallylglycine, crotylglycine, were prepared by reduction of oximes alkenylmalonates followed by hydrolysis.

(iv) By Reduction of Oximes of Barbituric Acid and Cyclopentanone

In 1864, Adolf Baeyer[60] treated barbituric acid with nitrous acid and obtained isonitrosobarbituric acid which on hydrolysis and reduction yielded aminomalonic acid.

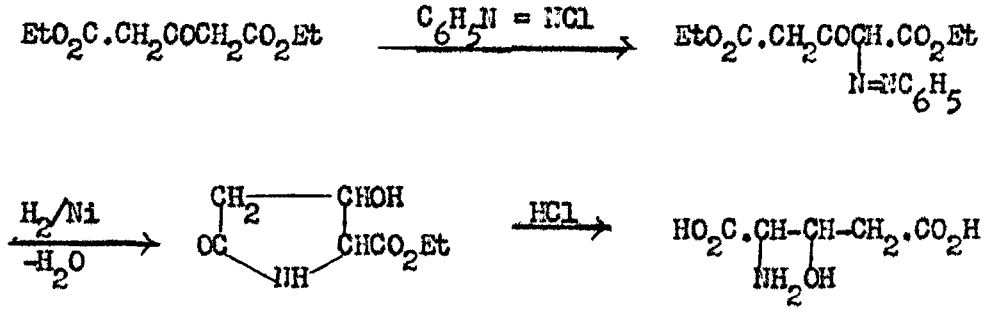


Fischer and Zemplen[61] prepared δ-aminovaleric acid and ornithine from cyclopentyloxime according to the following scheme:

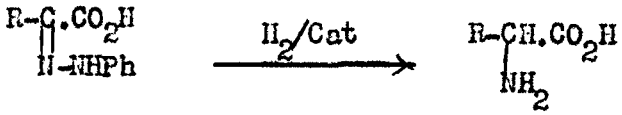


(c) Synthesis of Amino Acids by Reduction of Phenylhydrazones of Keto Acids or their Esters

The Japp-Klingemann reaction[63] provides a very good method for the preparation of phenylhydrazones of keto acids, acetoacetic, malonic and cyano esters, and 3-carboxypiperiodone which on subsequent reduction and hydrolysis yield amino acids in good yields. Izumi and Konishi[64] synthasised β-hydroxyglutamic acid starting from diethyl acetone dicarboxylate and benzene diazonium chloride.



Khan and Kidwai[65] prepared a number of amino acids by reductive cleavage of phenylhydrazones of keto acids.

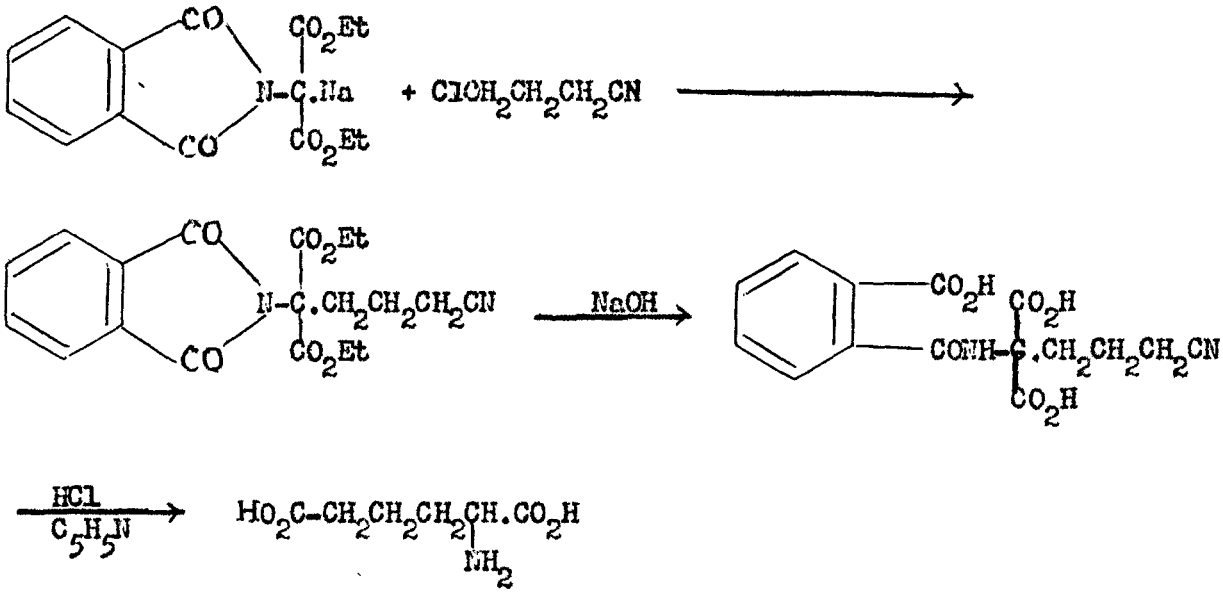


Using this method, other workers have prepared β,β-dimethylaspartic acid[66], ornithine[67], α,α'-diaminosuccinic acid[68], α-aminoadipic acid[69] and α-aminopimelic acid[70].

IV. METHODS BASED ON THE USE OF AMINOMALONIC ESTER AND RELATED COMPOUNDS

(a) Synthesis of Amino Acids by Phthalimidomalonic Ester Method

In 1903 Sorensen[20] developed a method for the synthesis of α-amino acids using phthalimidomalonic ester as starting material. He condensed ethyl sodium phthalimidomalonic ester with γ-chlorobutyronitrile to obtain ethyl phthalimidobutyronitrile malonate which on saponification followed by hydrolysis produced α-amino adipic acid.

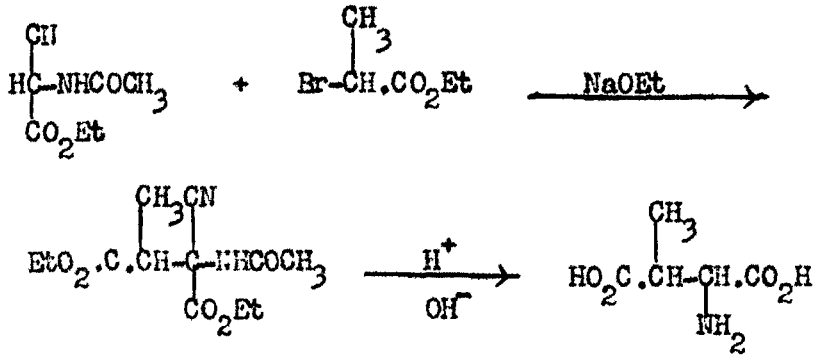


Other amino acids prepared by this method were α,α-diaminoadipic and pimelic acids[71], hydroxyornithine[72], hydroxynorvaline[73], homoserine[71], lanthionine[74], δ-methylthionorvaline[75], Pyridylalanines[76], kynurenine[77], O-methylserine[78] and 3,4-dihydroxyphenylalanine[79],

The main drawback of this method is that it is difficult to remove the phthaloyl residue in some cases.

(b) Synthesis of Amino Acids by Acetamidocyanoacetate Method

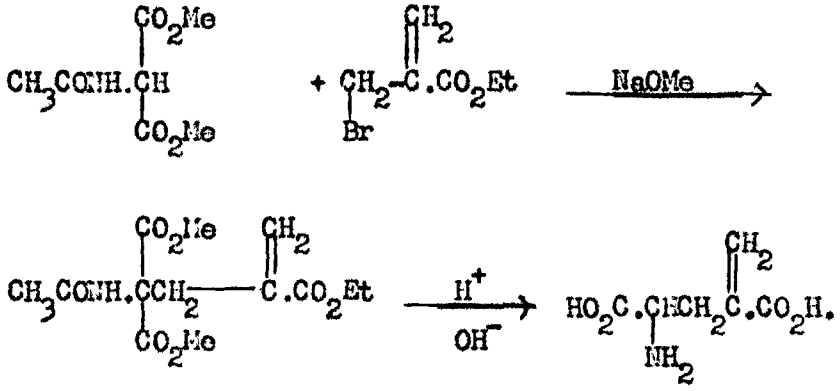
Winitz and Greenstein[80] synthesised β-methylaspartic acid in 50% yield by condensing ethyl acetamidocynoacetate with ethyl-α-bromo-propionate and hydrolysing the condensed product with hydrochloric acid.



Lin et al.[81] synthesised a good number of amino acids using different alkyl halides. Gaudry[82] used ethylamidocyanoacetate for the synthesis of ornithine. Ohta[83] employed this procedure in the synthesis of norleucine, norvaline and α-aminobutyric acid. Synthesis of 4-carboxy-tryptophan[84], α-amino-2-cyclohexenylacetic acid[85], α-aminotricarballylic acid[86], and 2-indanoglycine[87] has also been carried out using this method.

(c) Synthesis of Amino Acids by Acetamidomalonic Ester Method

In 1944 Snyder et al[88]. introduced acetamidomalonic ester for the synthesis of α-amino acids. Employing this method Hellmann and Lingens[89] prepared γ-methyleneglutamic acid by treating dimethyl acetamidomalonate with ethyl-α-(bromomethyl)-acrylate in presence of sodium methoxide and hydrolysing the product with hydrochloric acid.



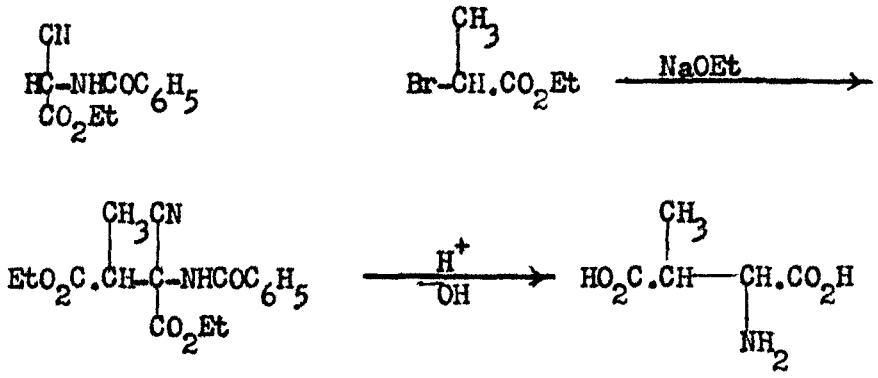
The other amino acids prepared by this method are α,α-diamino suberic and azeleric acids[90], α-methylproline[91], α-amino-β-(β-chloroethoxy) propionic acid[19], γ-oxalysine[19], β-phenylmethionine[92], O-[p-di(2-chloroethyl) aminophenyl] tyrosine[93], thiohistidine[94], N-methylamino acids[95], γ-methylglutamic acid[96], sulfur analogs of phenylalanine[97] and butyrine[98].

Using the above method a series of phenylalanine analogs has been synthesised by Burckhalter and Stephens[99]. Substituted derivatives of 3,4-dihydroxyphenylalanine[100], proline and glutamic acid analogs[101], 5-hydroxy-2-methyltryptophan[102]; 3-cumaronyl[103]-, p-guanidinophenyl-[104], 1-naphthyl-[105] and 9-phenanthryl-[105] analogs of alanine have also been synthesised recently.

The main advantage of this method is that the removal of acetyl group from the final product is much easier than the other groups.

(d) Synthesis of Amino Acids by Benzamidomalonic Ester Method

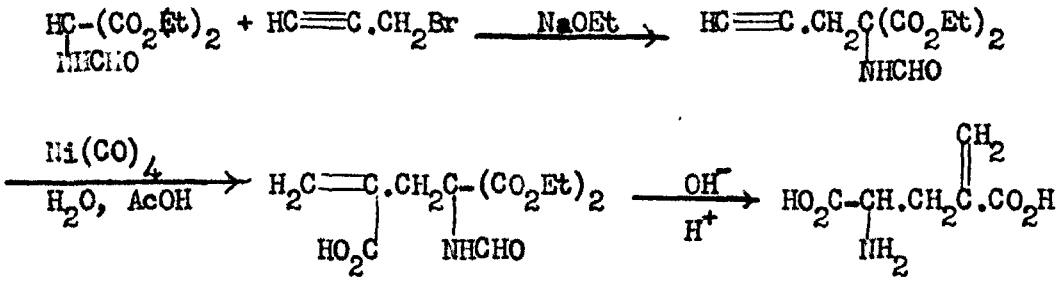
Dakin[106] synthesised β-methylaspartic acid by condensing diethyl-benzamidomalonate with α-bromopropionate followed by hydrolysis of the condensed product in 41% yield.



Pyridylalanines[107,108] have been synthesised similarly. Main advantage of benzamidomalonic ester method over the phthalimidomalonic ester method is that the substituted benzamidomalonic esters can be readily hydrolysed and decarboxylated to give amino acids.

(e) Synthesis of Amino Acids by Formamidomalonic Ester Method

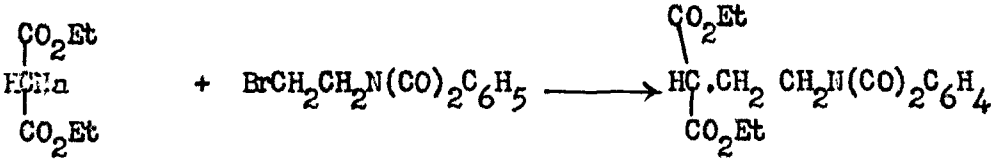
In 1947 Galat[109] employed this method for the synthesis of α-amino acids. He synthesised γ-methyleneglutamic acid by treating formamidomalonic ester with propargyl bromide to yield diethyl propargylformamidomalonate which on treatment with nickel carbonyl in aqueous ethanolic acetic acid followed by hydrolysis produced the amino acid.

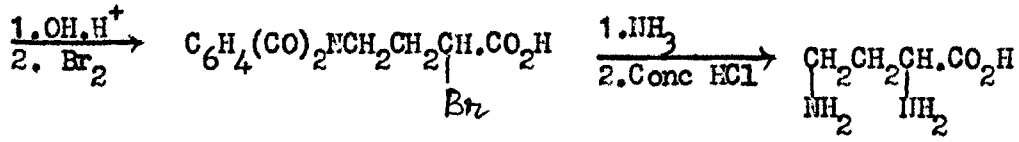


Dobson and Raphael[110] prepared baikiain, the imino acid isolated from Baikiaea plurijuga and dates, in 6 per cent yield. Avakian and Coworkers[111] used this method for the synthesis of 3-thionaphthenylalanine.

(f) Synthesis of Amino Acids by Malonic Ester and Substituted Malonate Method

Fischer[19] in 1901 for the first time synthesised γ-aminobutyrine, which is present in antibiotic substances polymyxin and aerosporin, starting from the sodium salt of malonic ester and bromoethylphthalimide.





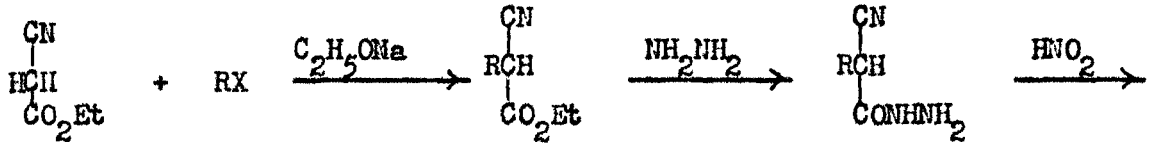
Rothchild and Fields[112] synthesised ornithine while Patterson and du Vigneaud[16] prepared homocystine. Traube et al.[113] obtained γ-hydroxyornithine as a side reaction on a synthetic route to hydroxyproline. Th s method has also been employed for the preparation of 5-hydroxytryptophan[114], α-fluoro and hydroxypyridylalanines[115], and 3-cumaronylalanine[116].

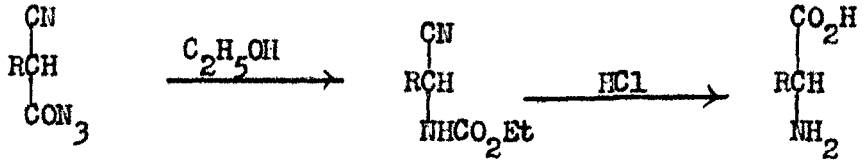
V. METHODS BASED ON THE USE OF AZIDE SYNTHESIS

(a) Synthesis of Amino Acids by Curtius Method

In 1921 Curtius and Sieber[117] developed a method for the synthesis of α-amino acids starting from substituted malonic esters. Alkyl substituted malonic esters were converted into alkyl malonylazidic acids and from them

the amino acids were prepared through the isatoic anhydrides, polymeric anhydrides or urethans. Gagnon and Coworkers[118-120] introduced the amides of ethylcyanoacetate for the synthesis of long chain, aliphatic α-amino acids.

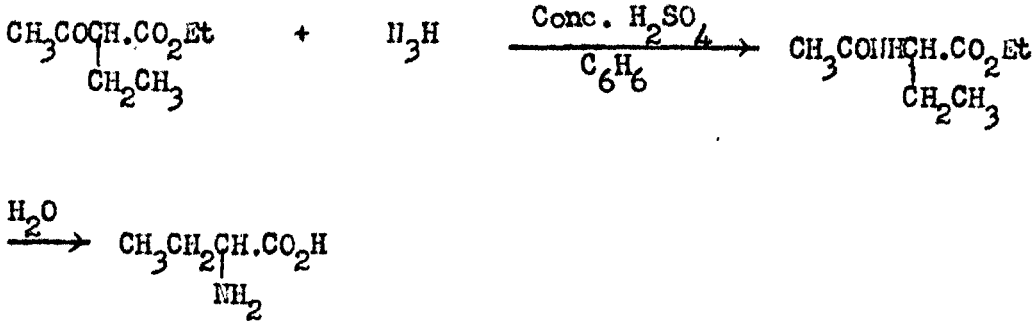




Preparation of γ-aminobutyrine[121] using this method has also been reported.

(b) Synthesis of Amino Acids by Schmidt Method

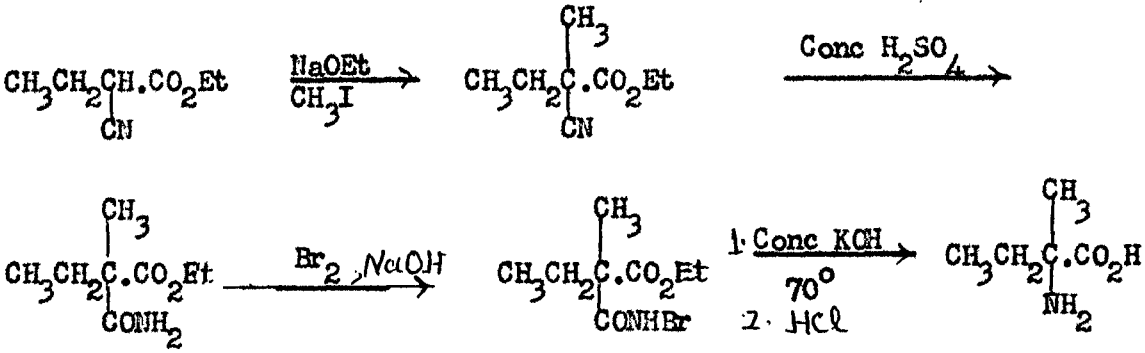
In1924 Schmidt[122] prepared a number of α-amino acids in excellent yields by the action of hydrazoic acid on substituted aceto-acetic esters followed by the hydrolysis of the resulting acetyl amino esters. Thus butyrine was prepared according to the following scheme:



Hayashi[123] subjected dialkyl substituted acetoacetic esters to Schmidt reaction and obtained a mixture of two or three amino acids in each case. Adamson[124] synthesised ornithine from cyclohexanone-2-carboxylic ester and cyclopentanone-2-carboxylic ester by a two fold Schmidt reaction.

(c) Synthesis of Amino Acids by Hofmann Degradation Method

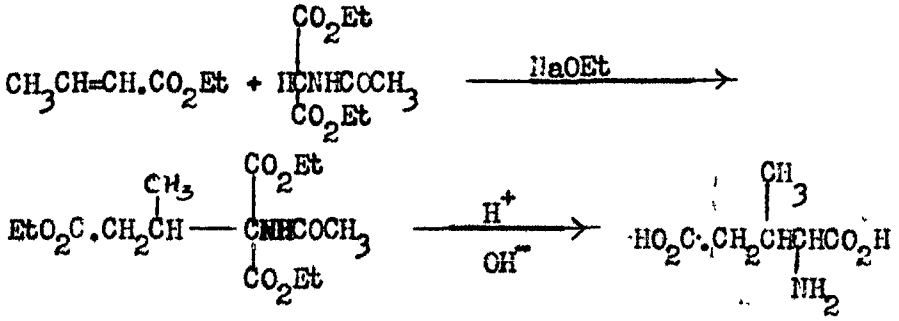
In 1942 Li et al[125] used Hofmann degradation for the synthesis of the α-amino acids and isovaline.



Karrer and Schlosser[126] prepared β-aminoalanine, found in Viomycin, from acetylasparagine. Huang et al[127] employed this method in the synthesis of decyline and δ-methyldecyline.

VI. METHOD BASED ON THE USE OF MICHAEL CONDENSATION

Morrison[128] in 1955 obtained a Michael condensation product on heating a mixture of ethyl crotonate and acetamidomalonate and the condensed product afforded β-methylglutamic acid on hydrolysis.

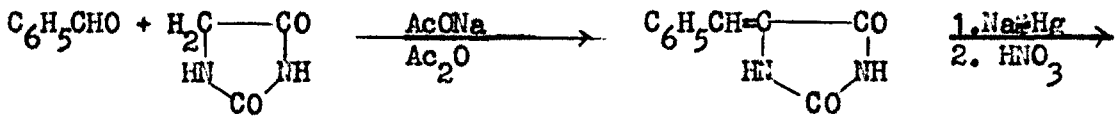


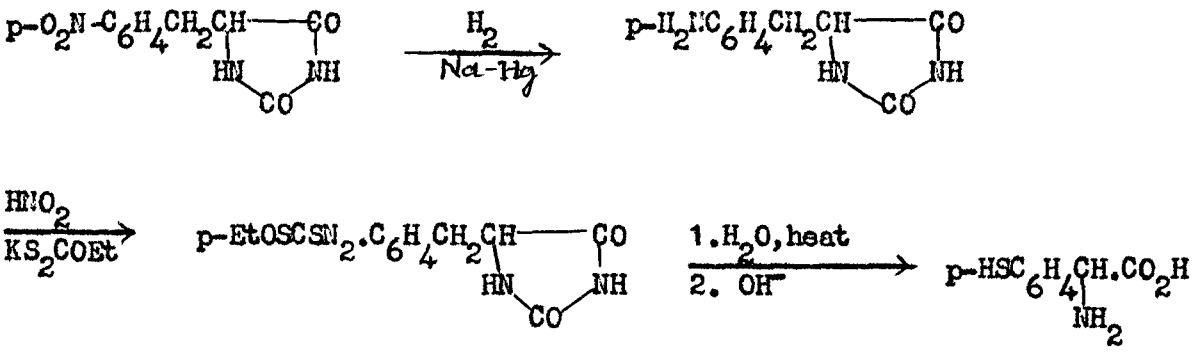
Harington[129] prepared β-phenylglutamic acid starting with ethylcinnamate and diethyl malonate. Atkinson and Poppelsdorf[130] did the synthesis of γ-aminobutyrine with acrolein and phthalimide. Synthesis of γ-methyleneglutamic acid[96], ornithine[131-132] and 1-hydroxyproline[133] was also done on similar lines.

VII. METHODS BASED ON THE USE OF CONDENSATION OF ALDEHYDES

(a) Synthesis of Amino Acids by Hydantoin Method

In 1911 Wheeler and Hoffmann[134] condensed aldehydes with hydantoin and converted the condensed products to α-amino acids in good yields. Johnson and Brautlecht[135] synthesised p-mercaptophenylalanine by condensing benzaldehyde with hydantoin to form benzalhydantoin which was thereupon converted to benzylhydantoin on reduction with sodium amalgam. On nitration the benzylhydantoin yielded the corresponding p-nitrobenzylhydantoin which on reduction with hydriodic acid formed p-aminobenzylhydantoin. This compound on diazolization and combination with potassium xanthogenate produced diazonium xanthogenate which on treatment with water followed by hydrolysis with barium hydroxide yielded the amino acid.

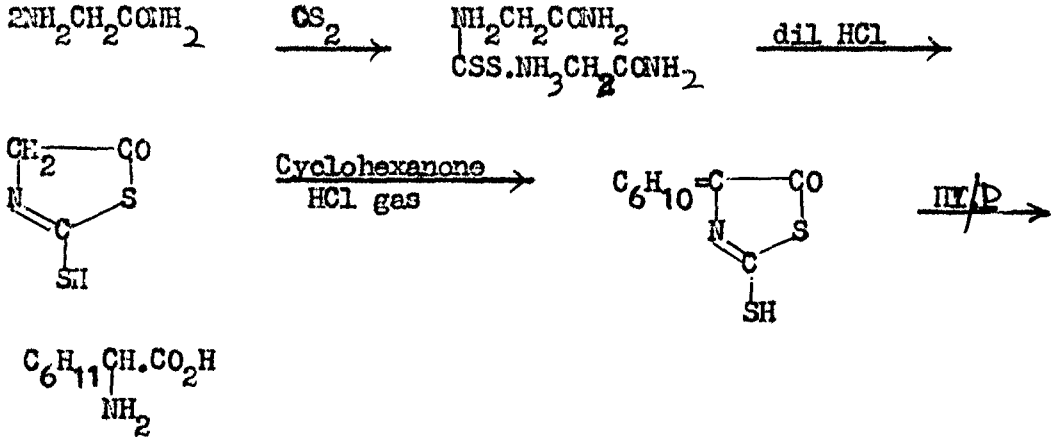




This method has been widely used in the literature for the synthesis of α-amino acids.

(b) Synthesis of Amino Acids by Thiohydantoin Method

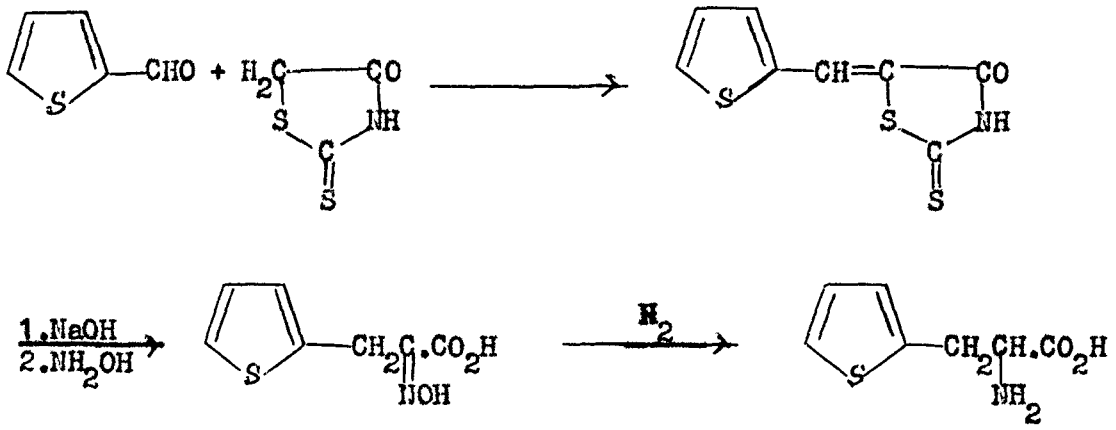
In 1911 Johnson and Nicolet[136] obtained 86% yield of 2-thio-3-benzoylhydantoin when potassium thiocyanate was heated with hippuric acid in the presence of acetic anhydride and glacial acetic acid. Billimoria and Cook[137] employed this method for the synthesis of cyclohexylglycine starting with glycine amide and carbon disulphide.



This method has also been applied in the synthesis of 4-hydroxy-1-naphthylalanine[138] and N-methyl- α-amino acids[139].

(c) Synthesis of Amino Acids by Rhodanine Method

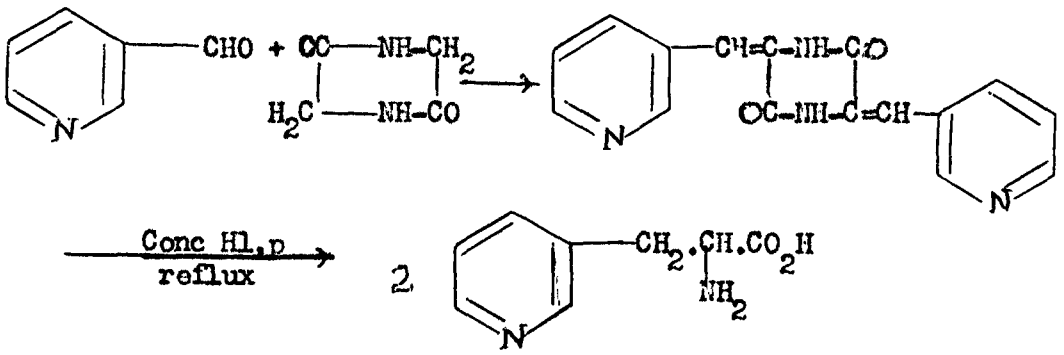
In 1903 anisalrhodanine was prepared by Andreasch and Zipser[149] and using this as a starting method Granschor et al[141] synthesised O-methyltyrosine. Crowe and Nord[142] condensed thienylaldehyde with rhodanine and obtained thienylalanine according to the following reaction scheme.



Some pyrimidine amino acids[143] have also been synthesised using the Rhodanine method.

(d) Synthesis of Amino Acids by Diketopiperazine Method

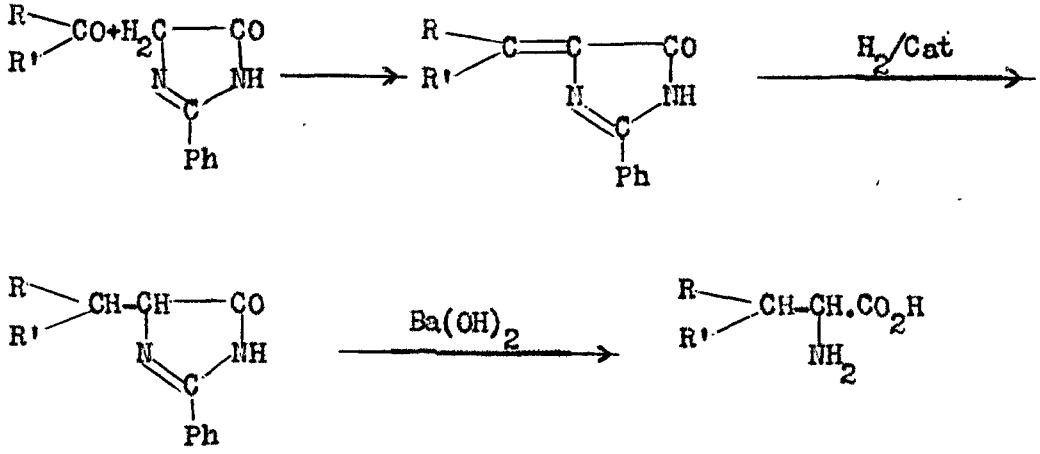
In 1921 Sasaki[144] developed a method for the synthesis of α-amino acids starting from glycine anhydride. Niemann et al.[107-8] prepared 3-pyridylalanine using this method.



Hirai[145] applied Sasaki's reaction to the preparation of 3;4-dihydroxyphenylalanine starting with vanillin and glycine anhydride along the similar line.

(e) Synthesis of Amino Acids by Imidazolone Method

Kidwai and Devasia[146] developed the imidazolone method for synthesis of α-amino acids. They condensed 2-substituted 5(4H)-imidazolones with carbonyl compounds to give unsaturated 2,4-substituted 5(4H)-imidazolones which on reduction followed by hydrolysis afforded amino acids.

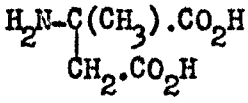


N; β-Dimethylleucine[147] was similarly synthesised.

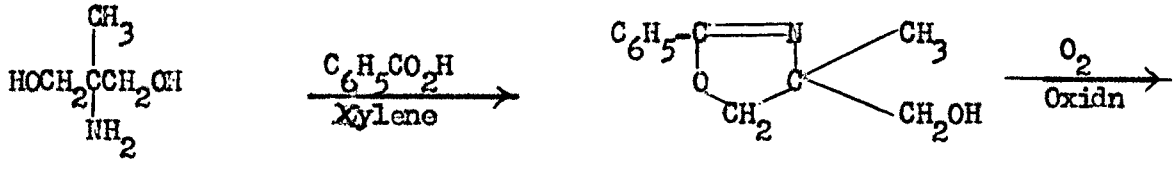
The aldehyde condensation method also covers the mercaptothiazolone method[137,148], the acetamidomalonic ester[149], the acetamidoacetic ester[150], the methyleneaminoacetonitrile[151] and the condensation of formaldehyde with glycine.[152]

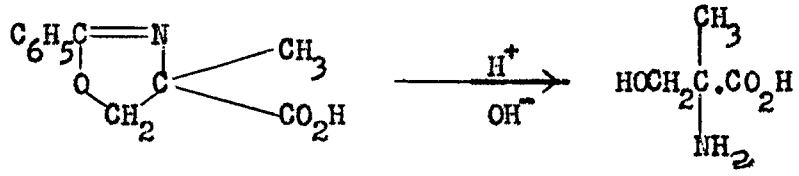
VIII. MISCELLANEOUS METHODS

Piutti[153] ammonolysed citraconic anhydride to yield methylasparagine which on hydrolysis gave α-methylaspartic acid.



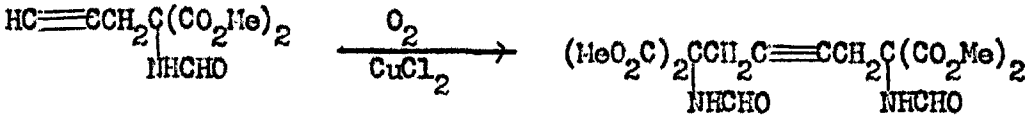
The preparation of aminotricarboxylic acid[154] was also effected by the alkaline hydrolysis of the diketopiperazine resulting from the action of ammonia on triethyl aconitate. α-Methylserine[155] has been synthesised by the reaction of 2-amino-2-methyl-1;3-propanediol with benzoic acid.

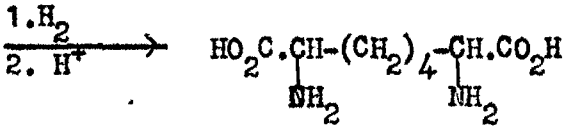




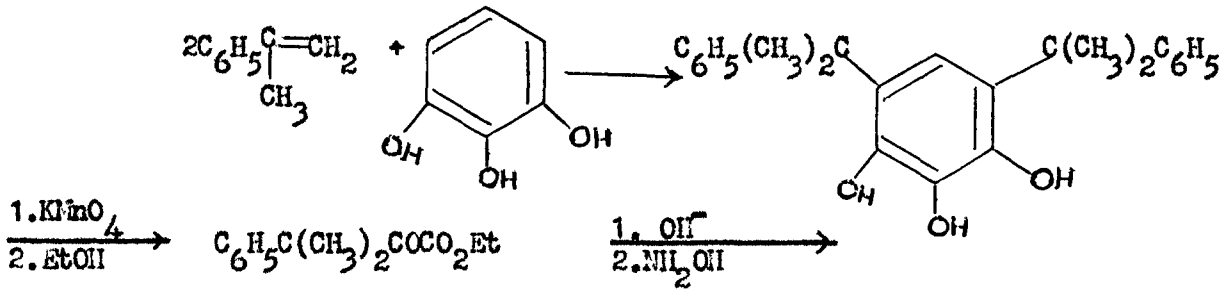
King and Kidd[156] obtained γ-aminobutyrine by the reaction of glutamic acid and phthalic anhydride. This amino acid has a strong physiological action on nervous and muscular tissues and depresses nervous and muscular activities. Plieninger[157] synthesised γ'-hydroxynorvaline by condensing oxalic ester with γ-butyrolactone. Smrt and Sorm[158] treated ethyl- α-acetylpropionate

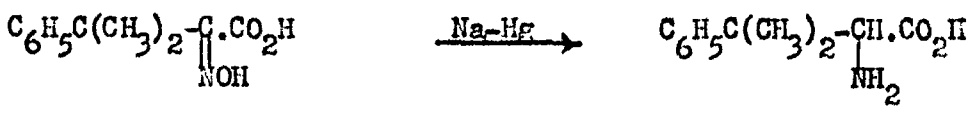
with ethyl- β-chloropropionate and then hydrazoic acid which they then hydrolysed to α-methylglutamic acid. Diaminosuberic acid[159] was also prepared by oxidative coupling of 2 moles of propargylformamide malonic ester followed by hydrogenation and hydrolysis.



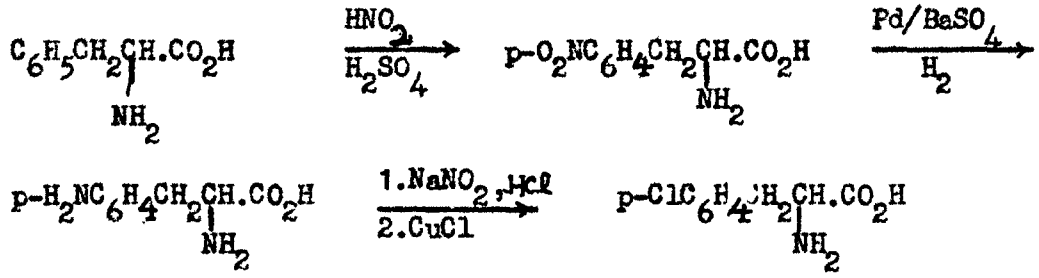


γ-Hydroxynorvaline and unsaturated α-amino acids were prepared starting from crotylglycine[160] and propargylglycine.[161] Wada[162] employed ornithine for the synthesis of citrulline, an amino acid isolated from the juice of watermelon. Lysine was converted to homoarginine by Groenstein[163] in 1938. Synthesis of baikiain also involved different procedures[164,109]. Jonsson[165] synthesised β-substituted amino acids starting from α-methylstyrene and pyrogallol as shown below





β-Methyltryptophan[166] was prepared by condensation of ethylidene isopropylamine with indole. Kjaer et al.[167] and Gaudry et al.[168] developed the synthesis of homomethionine which were based upon the reactions of allyl bromide with dimethyl formidomalonate and γ-bromopropyl hydantoin with methylmercaptan respectively. Canaline[169] and canavanine[170], both occur naturally in jack been, were obtained employing homoserine as the starting material. Akabori[171] in 1933 prepared 4(5)-methyl-2-thiolhistidine starting from alanine methyl ether. Bergel et al.[172] synthesised p-substituted phenylalanine nitrogen mustards starting from phenylalanine. 2-Bromopropane was used in the synthesis of hypoglycine by Carbon et al[173]. Houghten and Rapport[174] demonstrated the synthesis of p-chlorophenylalanine as shown below.



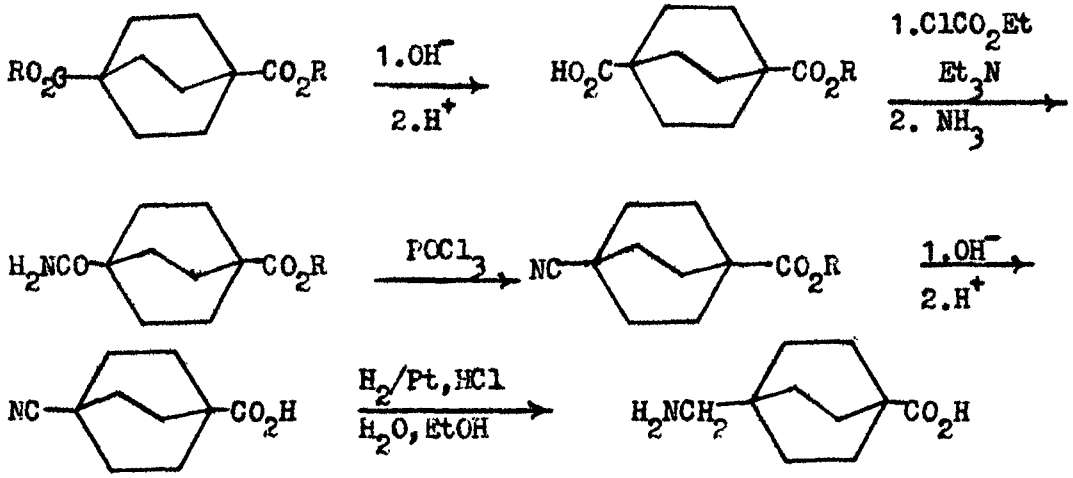
Fluorine analogs of amino acids preparation was reported by Lazar and Sheppard[175]. Hexafluoroleucine was synthesised by them as given below.

$$(CF_3)_2CHCH_2.CO_2H \xrightarrow[2.TosCl\text{-}py]{1.LAH} (CF_3)_2CHCH_2CH_2OTos$$

$$\xrightarrow{NaCN\text{-}DMSO} (CF_3)_2CHCH_2CH_2CN \xrightarrow[2.Br_2]{1.HCl} (CF_3)_2CHCH_2\underset{Br}{CH}.CO_2H$$

$$\xrightarrow[3.H_3O^+,py]{1.NaN_3\text{-}aqEtOH \\ 2.H_2/Pd\text{-}C} (CF_3)_2CHCH_2\underset{NH_2}{CH}.CO_2H$$

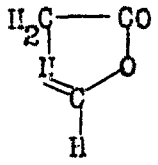
Besides these, a number of halo, seleno, pyrimidinyl, purinyl, purine and pyrrole substituted analogs of amino acids have been prepared using their own methods. Synthesis of α-amino acids was also effected with aminomalonic ester[176], phenylacetamidocyanoacetic ester[177], nitroacetic and nitromalonic ester[178] and amino alcohol oxidation[179] methods. Loeffler et al.[180] synthesised bridged bicyclic and polycyclic amino acids, the new inhibitors of the fibrenolytic process.



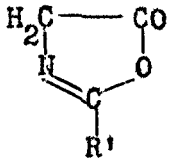
Kori and Takuhisa[180] demonstrated preparation of γ-aminobutyric acid starting from γ-butyrolactone. This amino acid is useful as antispasmodic and sedative.

IX. METHODS BASED ON THE USE OF AZLACTONES

Azlactones are derivatives of 2-oxazoline-5-one (I) with or without substitution at position 2 and 4. They are classified into two groups, saturated (II) and unsaturated (III).



(I)



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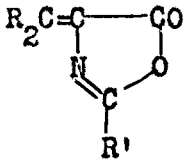


(III)

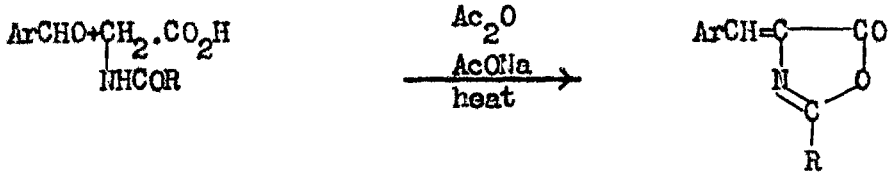
1. Synthesis of Azlactones

Several methods are available for the synthesis of unsaturated and saturated azlactones. They have been reviewed by Carter[181]. A brief account is given below:

(a) Preparation of Unsaturated Azlactones

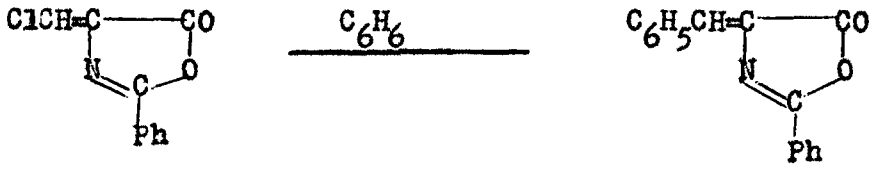
Preparation of the first unsaturated azlactone was reported in 1883 by Plochl[182], who condensed benzaldehyde with hippuric acid in the presence of acetic anhydride. However, the reaction of an aldehyde with

acylglycine in the presence of acetic anhydride and sodium acetate as a base catalyst is usually referred to as the Erlenmeyer Azlactone reaction[183].

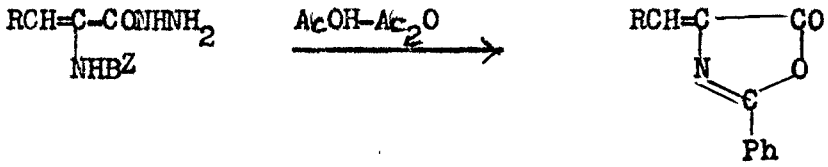


The intermediate in this reaction contains an active methylene group and therefore, the azlactone is formed by the action of acetic anhydride on acylglycine. Thus condensation takes place between an aldehyde and the azlactone rather than the acylglycine. Yields of the azlactones were improved using different metallic catalysts[184-189] in place of sodium acetate.

Action of acetic anhydride on an α-acylamino- β-hydroxy (alkoxy or acyloxy) acids[183], α-(α'-haloacyl)amino acid[189], and acyldehydroamino acids[190] in presence or absence of a base catalyst produced unsaturated azlactones. Azlactones are also obtained by the reaction of dialkyl or diarylformamidine with acylglycine[191], hippuric acid with ethylorthoformate[192], α-alkyloximino acids with an arylaldehyde[193] and hippuric acid with isoxazolium salts[194] in presence of acetic anhydride. Behringer[195] prepared unsaturated azlactones from 2-aryl-4-halomethylene-5-oxazolones by reaction with organometallic compounds, compounds with an acidic H and compounds which undergo Friedel-Craft's reaction.



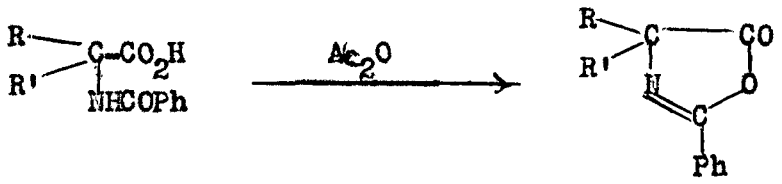
N-Acylamino acids react in presence of triethylamine with ethoxy carbonyl chloride and dicyclohexylcarbodiimide to form azlactones[196]. Synthesis of azlactones has also been effected by cyclodehydration with SO_3-complexes[197]. Bodea et al[198] obtained azlactones by the cyclization of α-acylamino hydrazides in the presence of acetic acid-acetic anhydride mixture.



Cyclization of α-haloacylamino acids to give azlactones has also been accomplished by treating the acid with benzoic anhydride and sodium benzoate at 100°C followed by distillation or treatment with $POCl_3$ and lutidine in CH_2Cl_2 at 15°C[199].

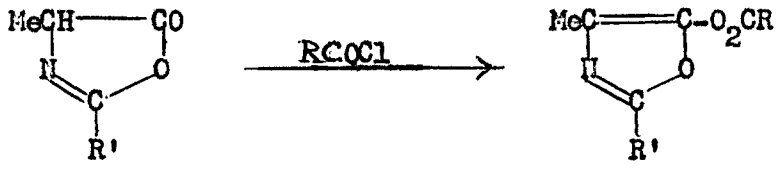
(b) Preparation of Saturated Azlactones

Mohr and Geis[200] in 1908 prepared the first saturated azlactone by heating acyl- α-amino isobutyric acid with acetic anhydride. In like manner, synthesis of saturated azlactones of other acyl-α-amino acids could be achieved by the action of acetic anhydride either alone or in acetic acid or pyridine[201] as a solvent.

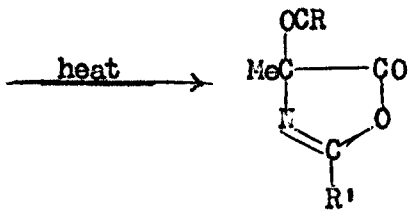


Azlactone formation was also effected by the reaction of silver compounds, e.g., silver benzensulfonate or silver oxide with phenylthioacetyl or thiobenzoyl derivatives of amino acids, hippuric acid with phosphorus tribromide followed by treatment with diazomethane[202], and by the reaction of alanine with trifluoroacetic anhydride and anhydrous trifluoroacetic acid to afford 2-trifluoromethyl-4-methyl-5-oxazolone[203].

Harry et al.[204] have successfully converted one azlactone into another by exposure to uv light in dioxane. Pino and Sletzinger[205] found that the reaction of acylchloride with 2-aryl-4-methyl-5-oxazolone (I) gave the ester (II) which rearranged on heating to the azlactone (III).



(I) (II)



(III)

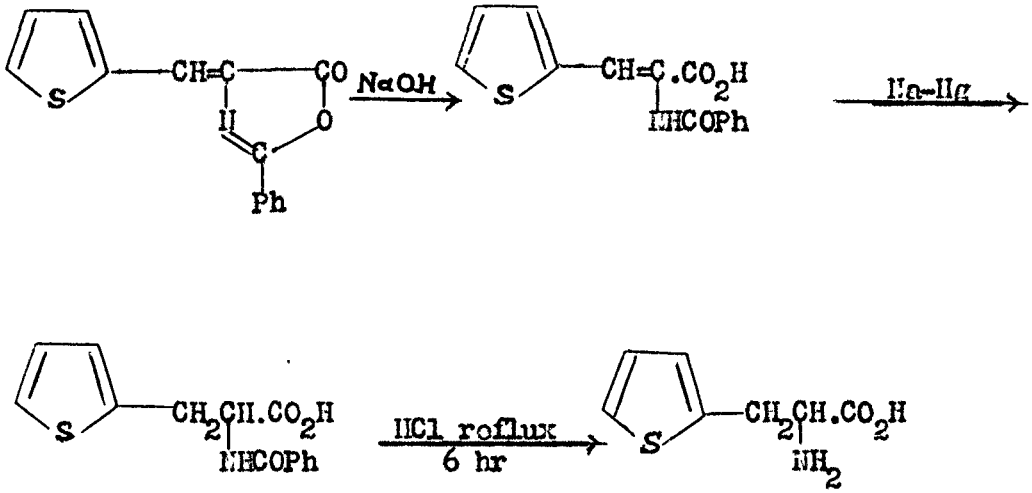
2. The Use of Azlactones in the Synthesis of α-Amino Acids

Erlenmeyer[183] in 1893 first employed azlactones as intermediate in the synthesis of α-amino acids. Published methods by which unsaturated azlactones and acylaminoacrylic acids can be converted into α-amino acids are summarized in the following pages.

(a) Preparation of Amino Acids by Chemical Reduction of Azlactones

(i) Reduction of Azlactones using Sodium or Sodium-amalgam and Water or Ethanol

Reduction of α-benzoylamino-acrylic acids with an equivalent amount of sodium amalgam (3%) has originally been described by Erlenmeyer[183]. Barger and Easson[206] prepared β-2-thienylalanine according to the following scheme:



A scrutiny of the literature reveals that the following amino acids have been synthesised by this method:

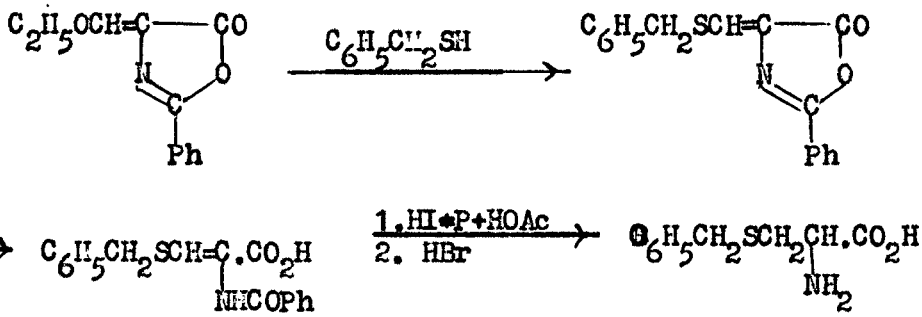
Amino Acids	% Yields	Ref
3/4-Methylphenylalanines	50,-	207,91
3/4-Chlorophenylalanines	37,78	208,209
4-Methoxy/Phenoxyphenylalanines	-	210,91
3;4/2; 4-Dimethoxyphenylalanines	-,62	211
3;4-Methylenedioxyphenylalanine	74	214
3-Hydroxy-4-methoxyphenylalanine	85	215
3-Methyltyrosine and o-tyrosine	65,-	213,216
1/2-Methyl and N-methyltryptophanes	40,60,-	217-218
Furfuryl/Piperonylalanines	-	211
γ-Histidine	-	219
Phenylacetylphenylalanine	95	226

The reduction with sodium amalgam is not always satisfactory. For example, 2-phenyl-4-(3',4',5'-trimethoxybenzal)-5-oxazolone is not reduced at all[221] and α-benzoylamino-β-(4-methoxy-1-naphthyl) acrylic acid gives only a 10% yield of the reduced product[222]. α-Benzoylamino-β-indoleacrylic acid[223,224] and α-benzoylamino-β-pyrroleacrylic acid[225] are also not satisfactorily reduced. A few improvements in this method of reduction have been tried. In an improved procedure[211], sodium salts of benzoylamino acrylic acids were reduced with excess of sodium amalgam to give α-benzoylaminopropionic acid in 62-80 percent yields. Reduction of α-benzoylamino-β-indoleacrylic acid is effected readily by the action of sodium and ethanol[224]. α-Benzoylamino-β-(N-methylindole) acrylic acid was reduced using sodium-lead alloy dissolved in ethanol[217].

Stewart and Woolley[228] synthesised α ;α'-diamino-β-hydroxypimelic acid, the amino acid which forms part of the structure of phytopathogenic toxin of Pseudomonas tabaci, by reduction of the β-group of the methylated 2-phenyl-4 (DL-γ-phthalimido-γ-carbomethoxybutyryl)-5-oxazolone with sodium borohydride followed by the hydrolysis of the reduced product.

(ii) Reduction of Azlactones Using Hydriodic Acid and Red Phosphorus in Acetic Acid or Acetic Anhydride

Harington and Barger[229] reported for the first time the use of a mixture of hydriodic acid and red phosphorus as a reducing agent for benzoylaminoacrylic acids to produce free amino acids directly. Yields were improved markedly by adding acetic anhydride[230] or acetic acid[231] to the reaction mixture. Azlactones can be used as such but better results have been achieved using acrylic acids or their esters. In 1951 Battazzi and Davis[232] synthesised S-benzylcysteine starting with 2-phenyl-4-ethoxymethylene-5-oxazolone



Hydroxybenzaloxazolones, which are destroyed by alkalies, are smoothly reduced by this method. Since good yields of α-amino acids are obtained by this procedure, therefore it has been widely used for their preparation.

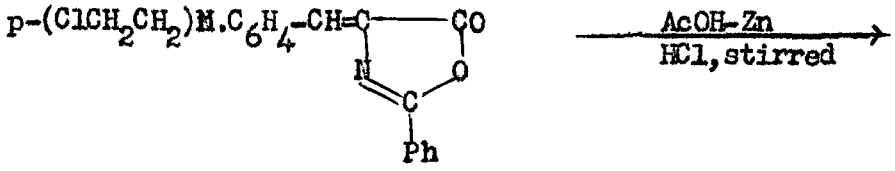
A survey of the literature shows that the following amino acids have been synthesised by this method

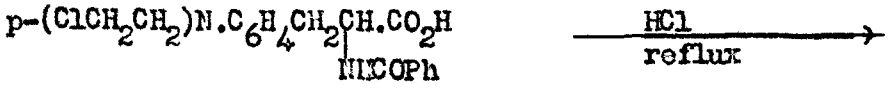
Amino Acids	% Yields	Ref
2/3/4-Fluorophenylalanines	37-78	208
4-(4'-Iodophenoxy/Aminophenoxyphenyl) alanines	-,62	210
Dihydroxyphenylalanines	-	211,213,233,234
3-Fluoro/3;4-Difluorotyrosines	60-80,13	213,236
2-or 3-Methyl/2;3-,3;5-,2;5-Dimethyl-tyrosines	61,62,65-72	213,237
2-Fluoro/2;5-Dichloro or Dibromo/ 3;5-Diodo-2'/3'/4'-thyronines	39,80,80 12-82	235,236,238 239,230
3'-Fluoro-3;4-diiodo/3;5-Diiodo-3';5'-difluorothyronines	42-60	235
Pyrenoylalanine	-	240
4-Methylthiazolyl-5-alanine	70	241
β-Mercaptophenylalanine	80	104
3-Thionaphthenylalanine	-	110
5-Methyl/2-thienylalanines	26.9,88.2	242
3-[8'-Quinolinol(5)-yl] alanine	96	243

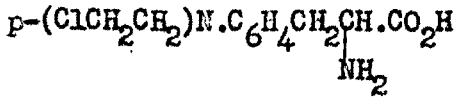
Main drawback of this method is that it involves some difficulties in the isolation of amino acids, also this method cannot be used for the synthesis of methoxy derivatives of amino acids.

(iii) Reduction of Azlactones using Zinc in Acetic Acid

Reduction of azlactones by stirring them with zinc dust in acetic acid has been reported by Konyukhov et al[244]. They prepared sacrolysine starting from 2-phenyl-4-(bis p-chloroethylaminobenzal)-5-oxazolone.



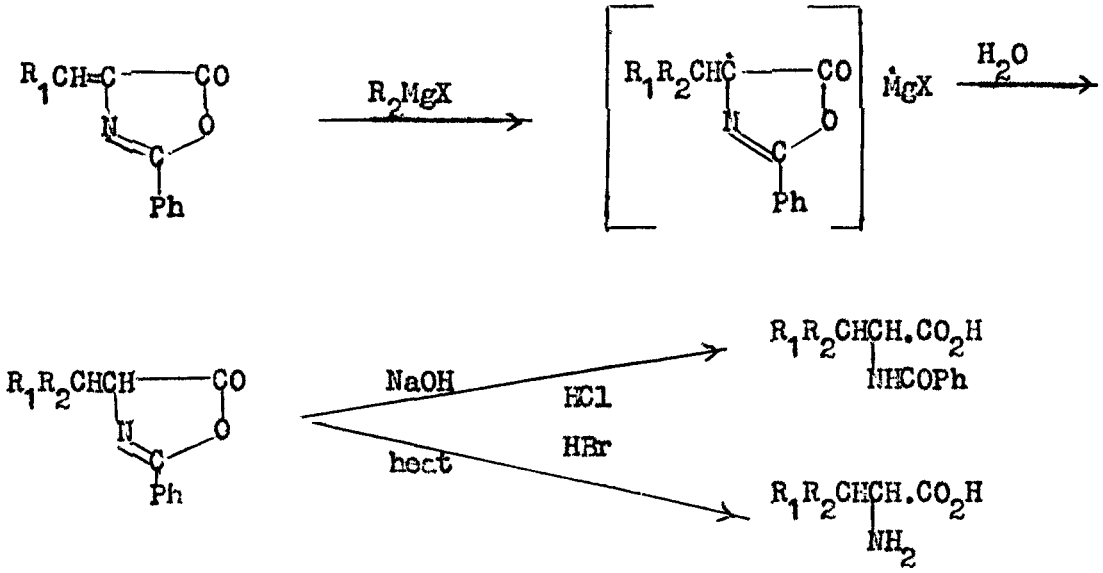




Similarly p- [bis(2-chloropropyl)amino] phenylalanine[245] and o-methoxy-p- [bis(2-chloroethyl)amino] phenylalanine[246] were prepared.

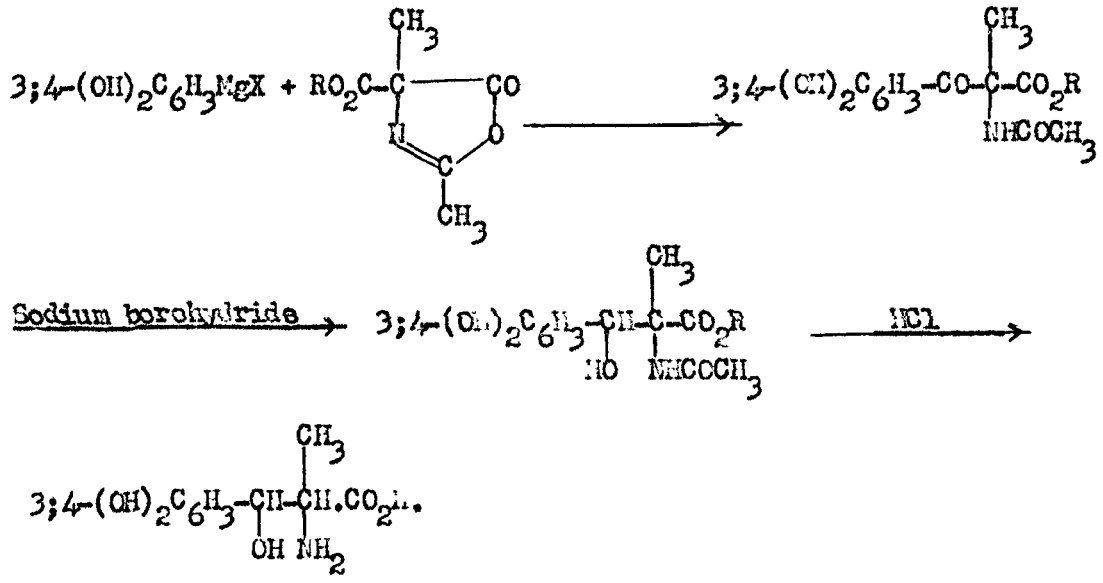
(b) Preparation of Amino Acids by the Reaction of Grignard Reagent with Azlactones

Horner and Schwahn[247] in 1955 prepared a number of β-substituted amino acids by treating azlactones with Grignard reagents to form the intermediate products by reaction at the carbon-carbon double bond which upon treatment either with alkali yielded the N-benzoyl derivatives or with 48% HBr gave the amino acids.



Using this method β-ethyl- β-dimethylalanine, β-ethyl- β-naphthylalanine and β-phenyl-β-(p-chlorophenyl) alanine were prepared in 75,80 and 90 per cent yields respectively while in other cases the yields were low.

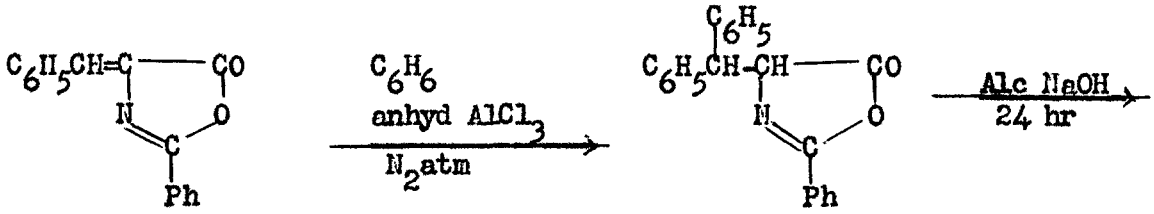
Pines et al.[248] synthesised 2-aryl-2-methylserines by the treatment of aryl Grignard reagents with azlactones. 3-(3;4-Dihydroxyphenyl)-2-methylserine was prepared in the following manner.

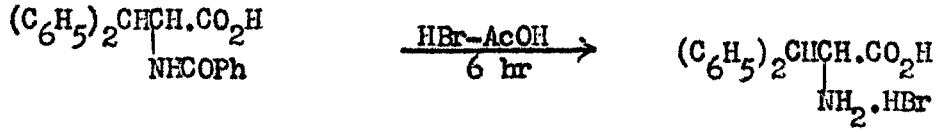


They synthesised erythro- and threo-derivatives of the amino acids.

(c) Synthesis of Amino Acidsby the Reaction of Azlactones with Benzene in the Presence of Anhydrous Aluminium Trichloride

Filler and Herbon[249] developed the reaction of benzene in presence of anhydrous aluminium chloride yielding 1,4-addition products, which on hydrolysis gave N-benzoyl derivatives and amino acids on treatment with alkali and hydrobromic acid respectively. They obtained $\beta;\beta$-diphenylalanine according to the following scheme.

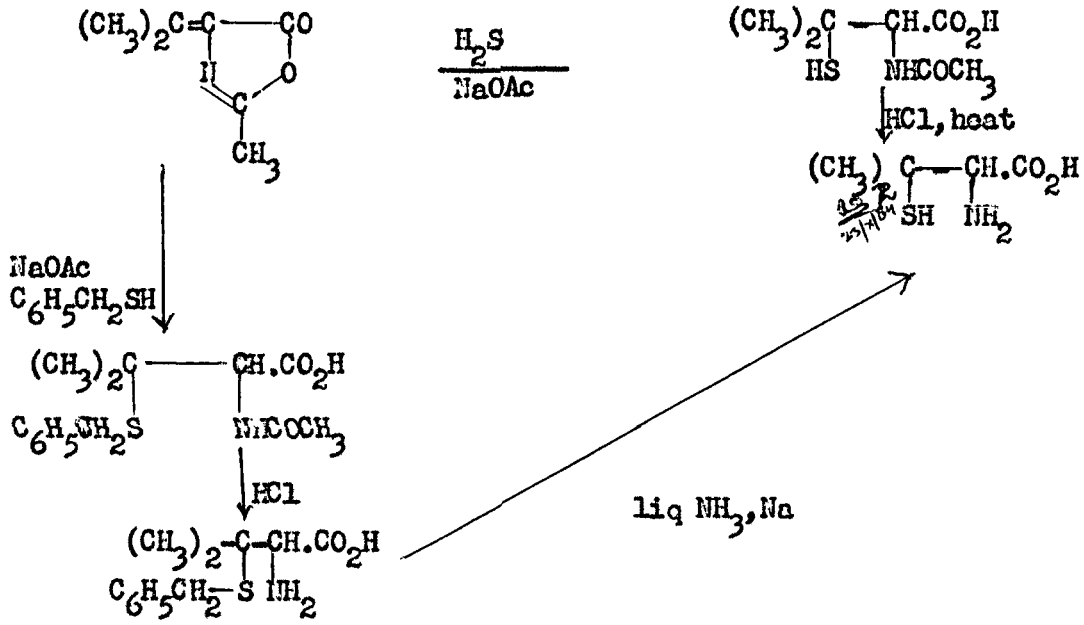




Similarly 2-chlorophenyl,2;4-dichlorophenyl, and 4-nitrophenyl substituted phenylalanines were prepared.

(d) Preparation of Amino Acids by the Reaction of Azlactones with Benzylmercaptan or Hydrogen Sulfide

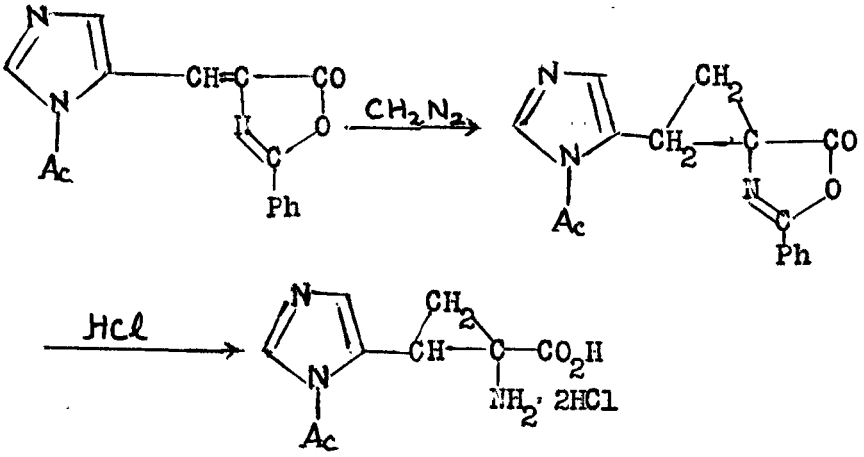
Nicolet[250] in 1932 treated 2-phenyl-4-isopropylidene-5-oxazolone with mercaptan to obtain penicillamine. This method was improved by other workers. They synthesised the amino acid[201] starting with 2-methyl-4-isopropylidene-5-oxazolone in place of its 2-phenyl analogs.



β-Thiolbutyrine[251], β-thiolnorvaline[101], β-thioliso leucine[201], β-thiolleucine[201] and S-methyl-β-phenylcysteine[92] were also prepared more or less by the same procedure.

(e) Preparation of Amino Acids by the Reaction of Azlactones with Diazomethane

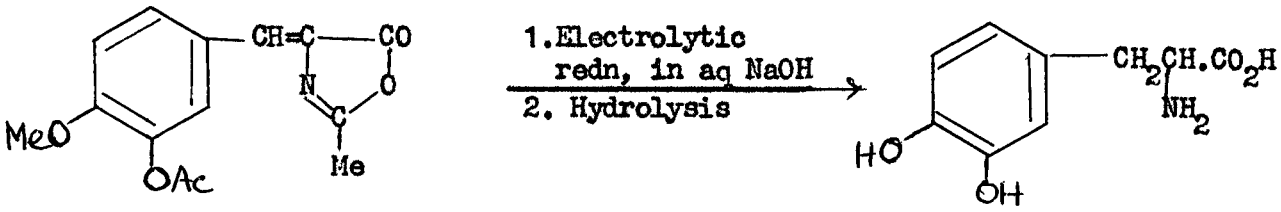
Pages and Burger[252] treated 2-phenyl-4-[(1-acetyl-4-imidazolyl) methylene]-5-oxazolone with diazomethane yielding the saturated azlactone which on hydrolysis gave 1-amino-2-(4-imidazolyl) cyclopropanecarboxylic acid.



Reaction of diazomethane with saturated azlactone, 2-methyl-4-acetyl-chloride-5-oxazolone, in the synthesis of 2-thiolhistidine was also reported[253].

(f) Preparation of Amino Acids by Electrolytic Reduction of Azlactones

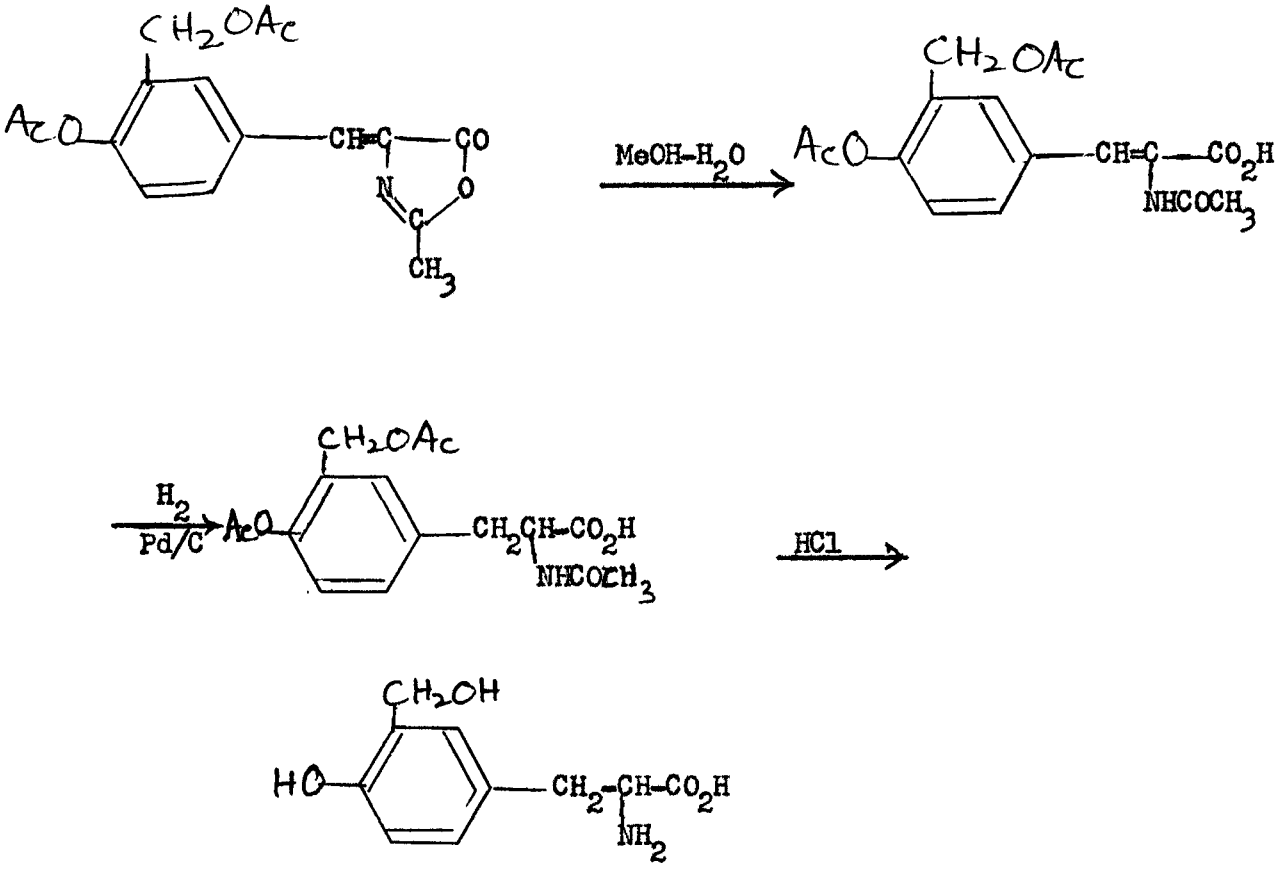
Recently 2-methyl-4-(3'-acetoxy-4'methoxybenzal)-5-oxazolone was reduced electrolytically in aqueous sodium hydroxide and 3;4-dihydroxyphenyl-alanine; an antipyretic or antiparkinsonism agent, was obtained by hydrolysing the reduced product[254].



(g) Preparation of Amino Acids by Catalytic Hydrogenation of Azlactones

Catalytic reduction has been used to a limited extent only. It is most satisfactory procedure except where other reducible or catalytic poisoning groups are present. Carter et al.[255] have reduced benzoylamino-crotonic acid azlactone smoothly over platinum catalyst and suggested that the saturated azlactone formed hydrolysed immediately.

Wang and Vida[258] hydrolysed the azlactone derived from chloromethyl aldehyde to give the unsaturated carboxylic acid. This was hydrogenated over palladium carbon to 3-(hydroxymethyl)tyrosine triacetate. Finally, hydrolysis of the triacetate produced 3-(hydroxymethyl) tyrosine in 71 per cent yield.



Amino acids reported in the literature by catalytic hydrogenation of azlactones are tabulated:

Amino Acids	Catalyst used	% yield	Ref:
3-Methoxy/3;-4-Dimethoxy phenylalanines	Rani Ni	84	256
Dihydroxyphenylalanines	Raney Ni, Pd/C	-	54,233
Furfuryl/3-Pyridylalanines	Pd/C, Raney Ni	92,73	256,257
δ-Phenylnorvaline	Raney Ni	96	256
3-Hydroxymethyltyrosine	Pd/C	71	258
3-Fluoro/3';5-Difluorothyronines	$Pd/CaCO_3$	-	235
α-Amino-β-hydroxystearic acid	Raney Ni	53	259
3;5;3'-triisopropylthyronine	-	-	260
3;4-Dihydroxyphenylalanine dimer	PtO_2	74	261

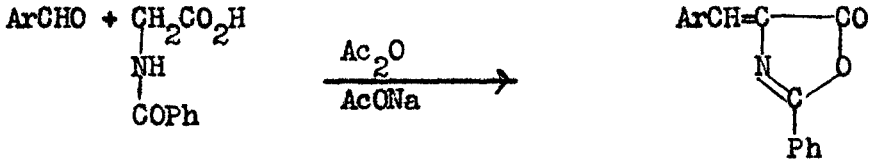
Catalytic hydrogenation could not be used in the synthesis of thyronine[229], reduction of pyrrole[185] and sulfur containing azlactones.

D I S C U S S I O N

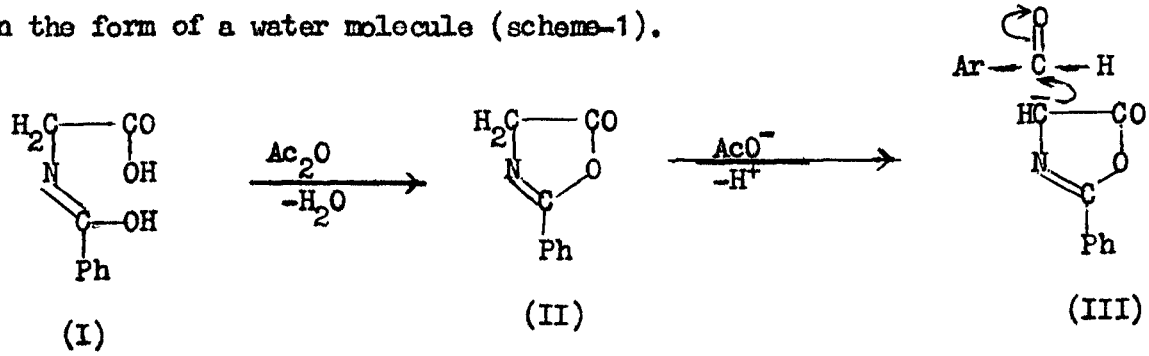
D I S C U S S I O N

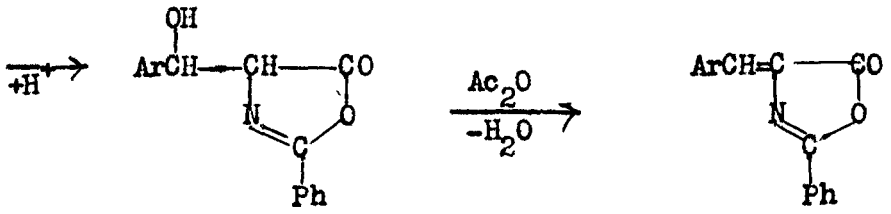
PREPARATION OF AZLACTONES

Plochl[182] prepared the first unsaturated azlactone by the condensation of benzaldehyde with hippuric acid in presence of acetic anhydride. However, it remained for Erlenmeyer[183] to determine the structure of the product. The reaction of an aldehyde with hippuric acid in the presence of acetic anhydride and sodium acetate is commonly referred as Erlenmeyer Azlactone Reaction (loc.cit).



The intermediate 2-phenyl-5-oxazolone (II), formed by the action of acetic anhydride on acylglycine (I), contains a methylene group which is doubly activated by the carbonyl group and the carbon-nitrogen unsaturated double bond. Condensation takes place between the aldehyde and the so formed azlactone (II) via (III) to yield 2-phenyl-4-(1'-hydroxybenzyl)-5-oxazolone(IV) which readily rearranges to 2-phenyl-4-benzylidene-5-oxazolone (V) by losing the hydroxyl group of the benzyl carbon and proton of the methylene group in the form of a water molecule (scheme-1).





(IV) (V)

Scheme-1.

For the preparation of azlactones we have tried a number of base catalysts, i.e., sodium acetate, potassium carbonate, lead acetate and potassium bicarbonate. A brief account of results obtained by us is given below.

Azlactones obtained from carbonyl compounds have been prepared by heating the carbonyl compound, hippuric acid and freshly fused sodium acetate with excess of acetic anhydride for varying lengths of time (15 min-2 hr). Table-1 shows the results obtained:

T A B L E - 1

Preparation of Azlactones using sodium Acetate

Carbonyl Compounds	Reaction time	% Yield Obtd.
1-Naphthaldehyde	1 hr	62.8
O-Methoxybenzaldehyde	30 min	75
β-Resorcylaldehyde	2 hr	91.2
Vanillin	15 min	75
p-Dimethylaminobenzaldehyde	20 min	69.2
Cyclopentanone	4.5 hr	33
Cyclohexanone	45 min	24.6

In the case of cyclopentanone, the ketone is added dropwise to the reaction mixture containing hippuric acid, fused sodium acetate and acetic anhydride and then it is refluxed. The azlactone of phthalic anhydride is also obtained by reacting with hippuric acid in the presence of this catalyst.

Galat[186] showed that potassium carbonate was an excellent catalyst for the condensation of aldehydes with hippuric acid and superior to sodium acetate. We used this catalyst in several preparations and obtained excellent yields. In such cases a mixture of an aldehyde, hippuric acid and potassium carbonate is stirred with acetic anhydride at room temperature. The reaction mixture set into a paste. The condensation takes place without external heating and is complete in a shorter period with appreciably higher yields than those obtained by the standard method. Douglas and Gulland[267] reported 77 per cent yield of the azlactone of m-nitrobenzaldehyde. The condensation of o-nitrocinnamaldehyde with hippuric acid to give the azlactone in 10 per cent yield was reported by Singhal and Ittyerah[268]. In our hands these aldehydes give almost quantitative yields of the azlactones when potassium carbonate is used. Crotonaldehyde yields 31 per cent of the azlactone when we take anhydrous sodium acetate. The yields is increased to 40 per cent when potassium carbonate is employed as a catalyst. Table 2 gives the results obtained

T A B L E - 2

Preparation of Azlactones using Potassium Carbonate

Aldehydes	% Yield Obtained
Salicylaldehyde	71
m-Nitrobenzaldehyde	100
o-Nitrocinnamaldehyde	98
Crotonaldehyde*	40

* The azlactone reported for the first time

Finar and Libman[184] reported the conditions underwhich aliphatic aldehydes were used in the Erlenmeyer azlactone synthesis and showed that much improved yields of the azlactones were obtained with lead acetate as the catalyst. We used this catalyst in the preparation of azlactones of aliphatic branched ketones. A mixture of lead acetate and hippuric acid is heated at reflux with a ketone and an excess of acetic anhydride. Results obtained are shown in Table-3.

T A B L E - 3

Preparation of Azlactones using Lead Acetate

Ketones	% Yield Obtained
Diacetone amine*	36.2
Mesityl Oxide*	34
Pinacolone*	42

* Azlactones reported for the first time

When cyclic ketones are used in this reaction the yields are much lower (8-10%).

We also used potassium bicarbonate as a catalyst. Condensation takes place similarly as in the case of potassium carbonate. The pure products are obtained directly in higher yields. The condensation of 3-pyridylaldehyde with hippuric acid to yield the azlactone was reported by Wibaut and Co-workers[279] in 58-67 per cent yield. The use of potassium bicarbonate permitted the reaction to occur at room temperature. In this way the azlactone is obtained in over 92.4 per cent yield. Carter[181] reported 67 per cent yield of the piperonal azlactone. We prepared this azlactone in 82 per cent yield by replacing the usual catalyst sodium acetate with potassium bicarbonate. The yield of crotonaldehyde azlactone remains unchanged with this catalyst (Table-4).

T A B L E - 4

Preparation of Azlactones using Potassium Bicarbonate

Aldehydes	% Yield Obtained
Pyridyl-3-aladhyde	92.4
Piperonal	82
Crotonaldehyde*	40

* The azlactone reported for the first time

Azlactones have usually been isolated either by cooling the reaction mixture and removing the azlactone by filtration or by pouring the cold reaction mixture into ethanol or water to allow excess of acetic anhydride to decompose and collecting the azlactone. In case of aliphatic ketones the azlactones have been extracted with boiling light pertroleum ether (bp 40-60°) after decomposition of acetic anhydride with water.

Most of the azlactones have been purified by recrystallisation from ethanol. Ethyl acetate is employed for the recrystallisation of o-nitro-cinnamaldehyde azlactone. The azlactones prepared from p-dimethyl-amino-benzaldehyde and cyclopentanone are recrystallised from benzene. The azlactone of vanillin is purified by recrystallisation from glacial acetic acid. Carbon tetrachloride is used for the recrystallisation of the azlactone of pinacolone. Certain solvent mixtures have also been used e.g., benzene-hexane and chloroform-hexane for the recrystallisation of azlactones derived from diacetone amine and mesityl oxide respectively.

REDUCTION OF AZLACTONES

A survey of the literature revealed that the unsaturated azlactones and their acylaminoacrylic acids were converted to α-amino acids by reduction and hydrolysis. Reductions were accomplished by chemical reduction with sodium[224], sodium amalgam[183-211] or sodium-lead alloy 217 in water or ethanol or sodium borohydride alone[228]. But chemical reduction was not always satisfactory as some azlactones were not reduced at all[221] while in other cases yields were often very low[222-224]. Hydriodic acid with red phosphorus in acetic acid or acetic anhydride[229-243] has also been employed for the synthesis of most of the amino acids. But this method caused some diffuclties as the isolation of amino acids is difficult and methoxy derivatives could not be synthesised by this method. Reduction with zinc in acetic acid[244-246] has also been used in a few cases. Thus sacrolysine[244], p-[bis(o-chloropropyl) amino] phenylalanine[245], and o-methoxy-p-[bis(2-chloroethyl)amino] phenylalanine[246] were synthesised by this procedure. Finally electrolytic reduction was used in the synthesis of 3;4-dihydroxyphenylalanine only[254] and catalytic hydrogenation followed by hydrolysis has been used to a limited extent[54, 256-261]. Platinum[261], palladium[233, 235, 257] and Raney nickel[54, 256, 259] were the catalysts so far employed for this purpose.

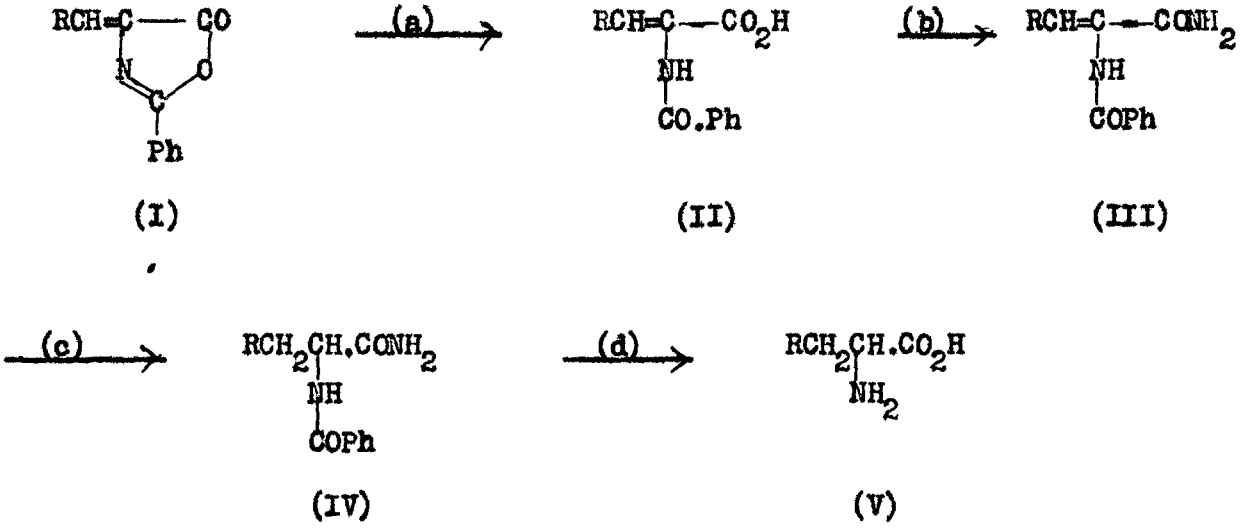
Published methods show that azlactones as such do not undergo catalytic reduction at low pressures of hydrogen (45-65 psi), but acylaminoacrylic acids and their amides can be reduced smoothly. The sequence of reactions leading to amino acids from azlactones generally involves four steps, i.e.,

(a) hydrolysis of azlactone (I) to acylaminoacrylic acid (II)

(b) conversion to unsaturated acylamino acid amide (III)[183,266,269]

(c) reduction to saturated acylamino acid amide (IV) and

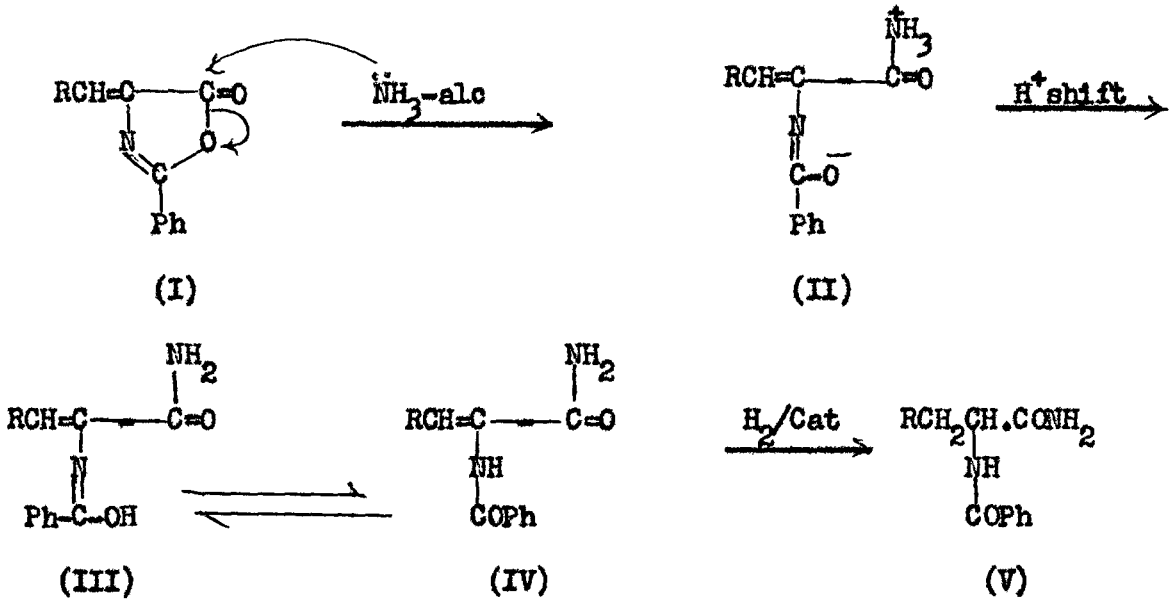
(d) hydrolysis to amino acid (V) (Scheme-2).



SCHEME -2

Isolation of intermediates at each step results in a lower yield of the amino acid. Therefore it was thought worthwhile to try to develop a method which combines high yields with few experimental operations. Since catalytic hydrogenation has not received sufficient attention, an attempt has been made to improve this method. It has been found that azlactones can be directly converted rapidly to the N-benzoylamino acid amides in high yield and greater purity in alcoholic ammonia in the presence of a catalyst under elevated hydrogen pressure and at room temperature. Thus the three steps mentioned above are combined in a one step operation.

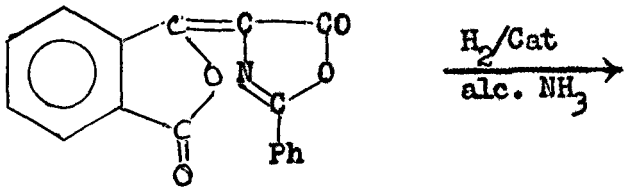
On the basis of experimental data we propose that the oxazolone ring (I) is first ammonolysed. This ring is opened by the attack of ammonia on the carbonyl carbon resulting in a charged species (II) in which proton shift takes place giving an enol (III). This intermediate then readily converts to the keto form N-benzoylacrylic acid amide (IV). Catalytic reduction of carbon-carbon double bond in IV leads to the formation of N-benzoylamino acid amide (V) as shown in Scheme-3.



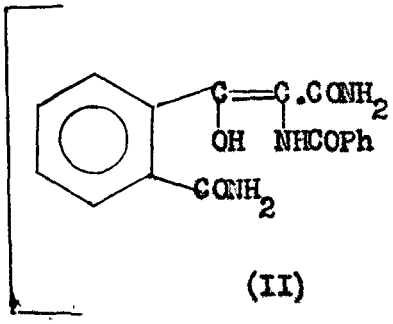
Scheme - 3

2-Phenyl-4-phthalidene-5-oxazolone(I)[183] reduction (Scheme-4) in this manner results in N-benzoyl-β-amino-β-(o-benzenecarbonamido) alanine amide (V) in which the lactone ring has also undergone ammonolysis alongwith 2-phenyl-5-oxazolone ring. The reaction is parallel to the ring opening of benzilidene phthalide through hydrolysis[270] and ammonolysis[271]. Gabriel[272] investigated that in the case of benzalphthalide, the lactone ring was opened by the attack of ammonia and not by the addition of ammonia to the ethylenic

linkage. In our reaction, the lactone ring is opened giving an enolic compound (II) which is converted to N-benzoyl-β-keto-β-(o-benzene-carbonamido) alanine amide (III), Reductive amination of this -keto compound(III) during hydrogenation gives the N-benzoyl- β-amino-β-(o-benzenecarbonamido) alanine amide (V) via (IV). If the azlactone is treated with ammonia alone, the intermediate N-benzoyl-β-keto- β-(o-benzenecarbonamido) alanine amide(III) is obtained which an subsequent hydrogenation in the absence of ammonia does not give the desired β-amino compound(V).



(I)



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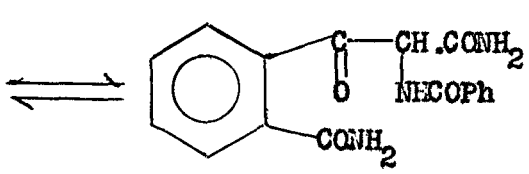
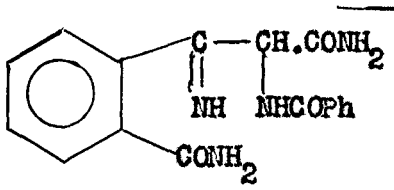
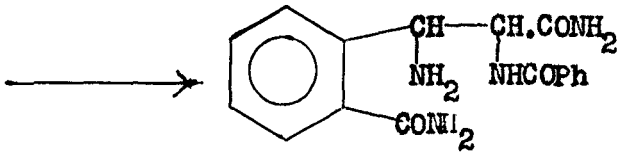


(III)



IV)



(V)

Scheme-4

IR spectra of 2-phenyl-4-phthalidene-5-oxazolone in KBr showed the characteristic absorption due to $\rangle C{=}O$ of 5-membered rings, $\rangle C{=}N-$ and $\rangle C = C\langle$ at frequencies 1810, 1670 and 1550 Cm^{-1} respectively. The reduced product N-benzoyl-β-amino-β-(o-benzenecarbonamido) alanine amide showed bands due to N-H at regions 3220 and 1630 Cm^{-1}, $\rangle \underset{|}{C} - N-$ bands at 1300 - 1260 Cm^{-1} and the $\rangle C = O$ frequency has been shifted to 1670 Cm^{-1} indicating that both the rings have been ammonolysed. No absorption band for ethylenic linkage was obtained showing thereby that the $\rangle C = C\langle$ bond has been saturated.

We have used Raney nickel to catalyse reduction of azlactones. In this case the azlactone is suspended in ethanol containing ammonia solution and freshly prepared Raney Nickel. This is reduced in a Paar Pressure catalytic hydrogenation apparatus under different hydrogen pressures for varying lengths of time to complete reduction. The filtrate and washings are evaporated to dryness under reduced pressure and the residue so secured is crystallised from a suitable solvent to give N-benzoylamino acid amide in sufficiently pure form and high yield.

Suspension of powdered azlactone in ammonical ethanol serves the purpose well and it is not necessary to have a clear solution of the azlactone before subjecting it to hydrogenation. Ammonia solution is taken in excess to suffice the amide formation. In most of our experiments, e.g., benzoyl-1-naphthylalanine amide, benzoyl-o-methoxyphenylalanine amide, benzoyl-2; 4-dihydroxyphenylanine amide, benzoyl-β-3-methoxy-

4-hydroxyphenylalanine amide, benzoyl-β-amino-β-(o-benzenecarbonamido) alanine amide, benzoyl-β-p-dimethylaminophenylalanine amide, benzoyl-cyclohexylglycine amide, benzoyl-o-tyrosine amide and benzoyl-β-piperonylalanine amide, the products crystallise out. This requires heating of the reaction mixture on a steam bath in order to dissolve it before the catalyst could be removed. In case of benzoylcyclopentylglycine amide, benzoyl-norleucine amide, benzoyl-δ; δ -dimethyl-ε-aminoisoleucine amide benzoyl-δ; δ -dimethylisoleucine amide and benzoyl-β; γ-dimethylleucine amide, the product is soluble in the reaction mixture but even then these are heated to boiling before removal of the catalyst and filtered. The catalyst is always washed thoroughly with boiling ethanol to free it from any adhering benzoylamino acid amide. Results of these experiments are shown in Table 5.

T A B L E - 5

N-Benzoylamino acid amides prepared by Raney nickel catalysed reduction of azlactons

DL-N-Benzoylamino Acid Amides	Hydrogen pressure Psi	Time hr	% Yield Obtd.	Mp °C recorded
DL-N-Benzoyl-β-1-naphthyl-alanine amide	47	10	90	221-22
DL-N-Benzoyl-β-o-methoxy-phenylalanine amide	45.8	11	61	220-21
DL-N-Benzoyl-β-2;4-dihydroxy-phenylalanine amide	42	7	78	286-87
DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine amide *	43	10	80	209-10
DL-N-Benzoyl-β-am no-β-(o-benzene-carbonamido)alanine amide	52	10.4	59	196-97
DL-N-Benzoyl-β-p-dimethylamino-phenylalanine amide	49.5	6	65	251-52
DL-N-Benzoylcyclopentyl-glycine amide	50	9	63	190-91
DL-N-Benzoylcyclohexylglycine amide	49	5	81	269-70
DL-N-Benzoyl-o-tyrosine amide	58	5.5	82	177-78
DL-N-Benzoylnorleucine amide **	39	3	62	161-62
DL-N-Benzoyl-δ;δ-dimethyl-δ-aminoisoleucine amide	46	5	55	145-46
DL-N-Benzoyl-δ;δ-dimethyliso-leucine amide	53	8	58	196-97
DL-N-Benzoyl-β;γ-dimethylleucine amide	38	6	62.4	166-67
DL-N-Benzoyl-β-piperonylalamine amide	42.8	8	82.5	215-16

* Lit 256 mp 209-10°, yield 83.5%

** Lit [273] mp 143-44°

The use of tetrahydrofuran or dioxane in place of ethanol as the reaction solvent minimizes the reduction time with little variation in the yield of the reduced product.

Azlactones of m-nitrobenzaldehyde, o-nitrocinnamaldehyde and pyridyl-3-aldehyde when subjected to prolonged Raney nickel catalysed hydrogenation, fail to yield a reduction product even when hydrogen pressures upto 62 psi and temperatures of 80°C are used. Attempts to hydrogenate these compounds using palladium-charcoal in presence of alcoholic ammonia at low hydrogen pressures however catalyse the reduction. This is unexpected as palladium charcoal is not effective in the reduction of azlactones in neutral medium. An important aspect of this procedure is its application to the facile reduction of other azlactones too. In all cases higher yields of the reduced products are obtained. The results are summarized in Table-6.

T A B L E - 6

N-Benzoylamino acid amides prepared by Palladium-charcoal catalysed reduction of azlactones

DL-N-Benzoylamino acid amides	Hydrogen pressure psi	Time hr	% Yield Obtd.	Mp °C recorded
DL-N-Benzoyl-β-1-naphthyl-alanine amide	36	8	95	221-22
DL-N-Benzoyl-β-o-methoxy-phenylalanine amide	41	9	77.4	220-21
DL-N-Benzoyl-β-2;4-dihydroxy-phenylalanine amide	38	5	78	286-87
DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine amide *	39.2.	6.5	85	209-10
DL-N-Benzoyl-β-amino-β-(o-benzenecarbonamido)alanine amide	43	8	64.5	196-97
DL-N-Benzoyl-β-p-dimethylamino-phenylalanine amide	40	4	71	251-52
DL-N-Benzoylcyclopentyl-glycine amide	45	8	67	190-91
DL-N-Benzoylcyclohexylglycine amide	38	5	86	269-70
DL-N-Benzoyl-o-tyrosine amide	49.6	4	86	177-78
DL-N-Benzoyl-β-m-aminophenyl-alanine amide	50	2.5	86	209-10
DL-N-Benzoyl-δ-o-aminophenyl-norvaline amide	47	1	98	150-51
DL-N-Benzoylnorleucine amide **	28	3	68	161-62
DL-N-Benzoyl-δ;δ-dimethyl-δ-aminoisoleucine amide	42	5	69	145-46
DL-N-Benzoyl-δ;δ-dimethyliso-leucine amide	39	5.2	68	196-97
DL-N-Benzoyl-β;γ-dimethyllecuine amide	32	5	65	166-67
DL-N-Benzoyl-β-3-pyridylalanine amide	36	7	95	189-90
DL-N-Benzoyl-β-piperonylalanine amide	40	5	85	215-16

* lit[256] mp 209-10°, yield 83.5%
** lit [273] mp 143-44°,

In actual practice an azlactone is suspended in ethanol containing ammonia solution and palladium charcoal (10% Pd). The reduction is carried out under different hydrogen pressures (28-50 psi) for varying lengths of time (1-9 hr). When the reduction is complete, the flask is disconnected, heated on a steam bath and filtered hot. The catalyst is washed thoroughly with boiling ethanol. The filtrate and washings are evaporated to dryness under reduced pressure and the residue so obtained is crystallised from a suitable solvent to give benzoylamino acid amide.

Reduction of azlactones is faster with higher yields (94.5-98%) when palladium charcoal catalyst is used whilst the reduction with Raney nickel under identical conditions takeslonger time (3-11 hr) and lower yields are obtained (55-90%).

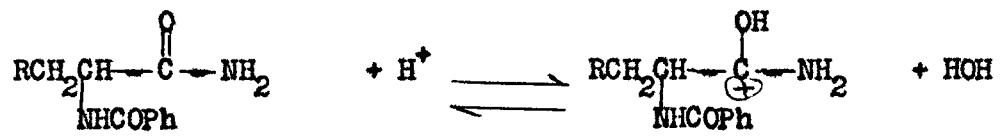
Most of the amides are purified by recrystallisation from ethanol(95%). Ethyl acetate is employed for the recrystallisation of N-benzoyl-δ-o-aminophenylnorvaline amide and N-benzoyl-δ; δ -dimethyl-δ-aminoisoleucine amide. N-Benzoyl-β-p-dimethylaminophenylalanine amide and N-benzoylnorleucine amide are recrystallised from glacial acetic acid and aqueous ethanol (30%) respectively. Certain solvent mixtures have also been used, e.g., ethanol-ethylacetate and benzene-methanol for the recrystallisation of N-benzoyl-β-3-pyridylalanine

amide and N-benzoyl-δ; δ -dimethylisoleucine amide respectively. Similarly recrystallisation of N-benzoyl-β; γ -dimethylleucine amide from ethyl acetate-chloroform mixture and N-benzoylcyclopentylglycine amide from benzene-ethanol mixture is effected.

HYDROLYSIS OF THE N-BENZOYLAMINO ACID AMIDES

Acid amides on treatment with an acid or alkali are converted to carboxylic acids. Thus N-benzoylamino acid amides are hydrolysed with an acid or alkali to the corresponding N-benzoylamino acids under mild conditions. Hydrolysis of the amides under reflux gives amino acids directly in excellent yields. The hydrolysing agents used are hydrochloric acid, hydriodic acid in presence of red phosphorus, sodium hydroxide and barium hydroxide.

On the base of our experimental work we suggest that in the case of N-benzoylamino acid amide (I) hydrolysis of the primary amide takes place first and then the secondary amide linkage is hydrolysed to yield the corresponding N-benzoylamino acid (V) and amino acid (VIII) respectively. It involves nucleophilic substitution, in which the amide group is replaced by a hydroxyl group. Under acidic conditions the amide is protonated to give II and water molecule is added resulting an intermediate III which gives N-benzoylamino acid (V) via IV by the elimination of one molecule of ammonia. Similarly hydrolysis of the secondary amide in V proceeds to produce amino acid (VIII) via VI and VII as given in Scheme-5.



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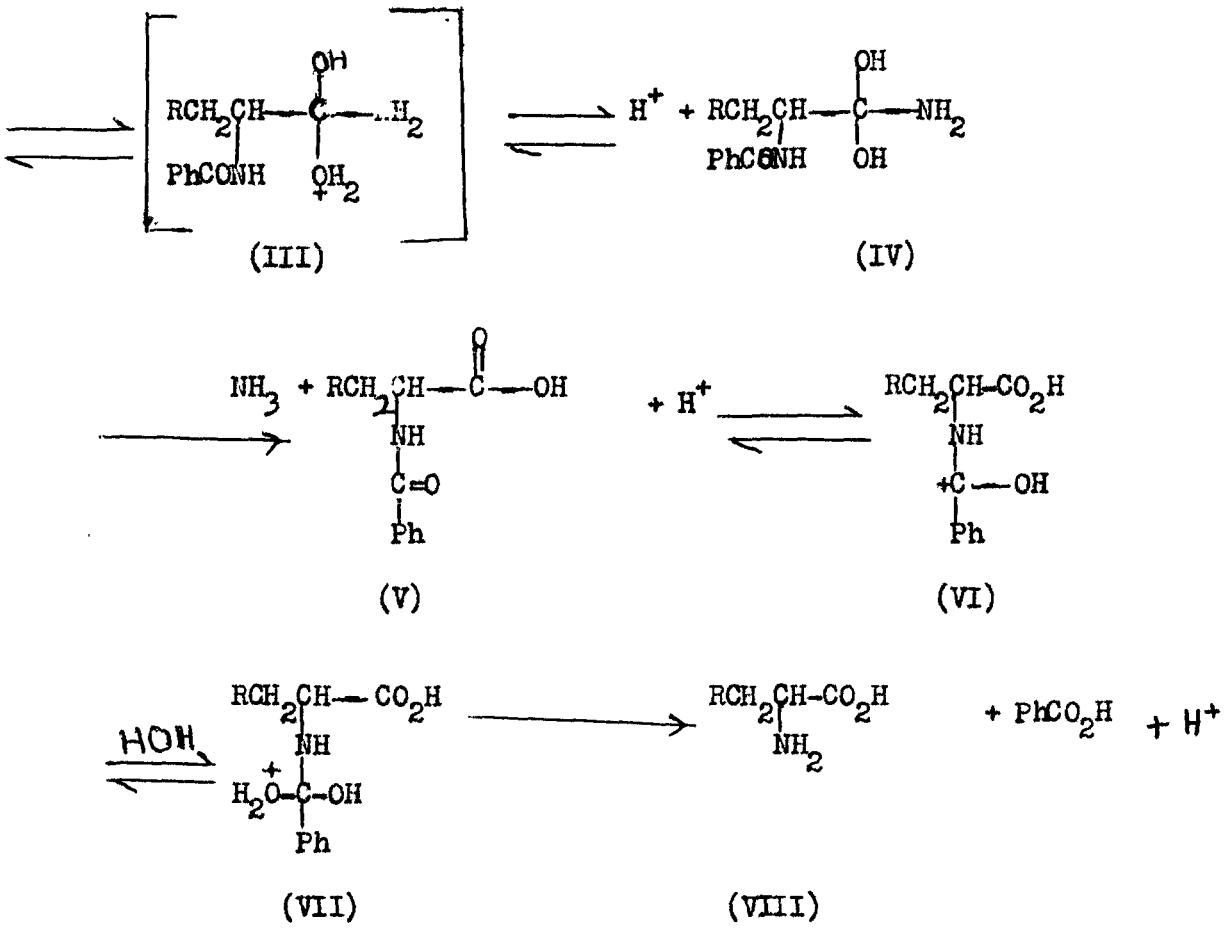
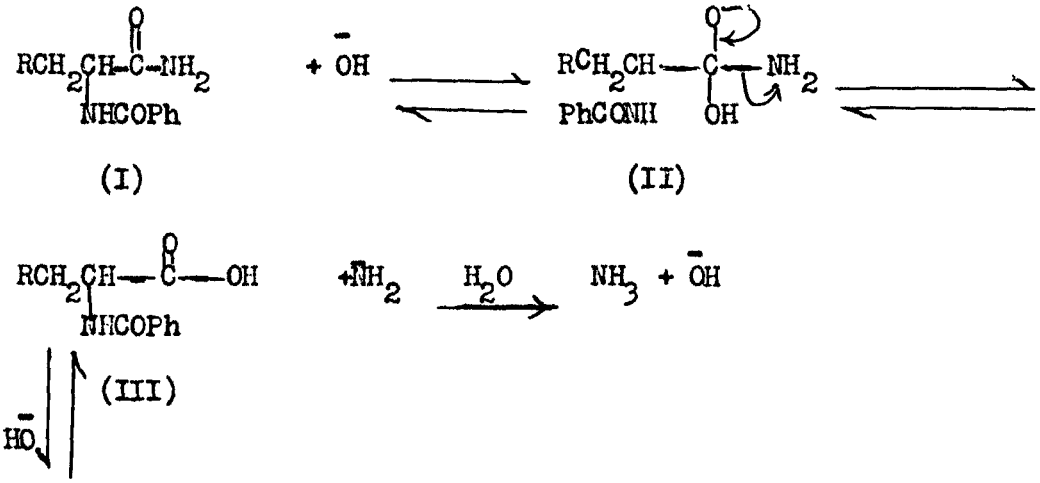
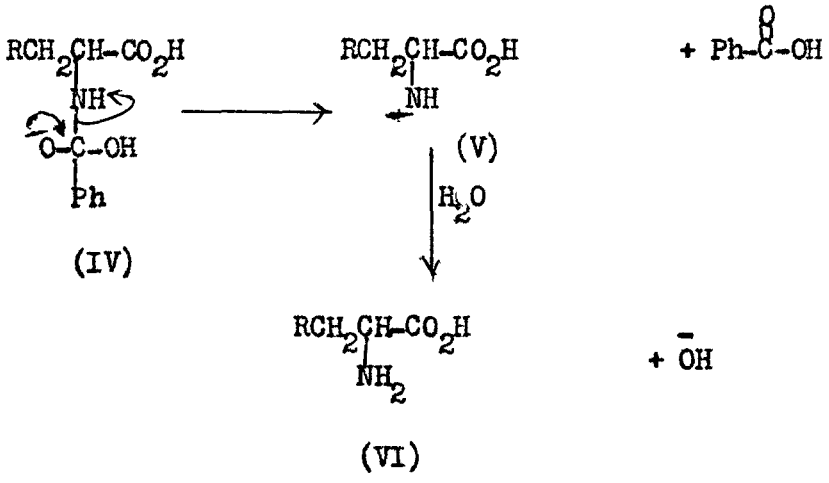


Scheme-5

Under alkaline conditions hydrolysis involves attack by the strongly nucleophilic hydroxide ion on the amide itself (Scheme-6).





Scheme-6

SYNTHESIS OF N-BENZOYLAMINO ACIDS

Different hydrolysing agents have been used for the preparation of N-benzoylamino acids. A brief account is given below:

Conversion of N-benzoylamino acid amides into corresponding N-benzoylamino acid is carried out by warming the former with hydrochloric acid (36%) until a clear solution is obtained (0.3-32 hr) and then allowing the reaction mixture to stand at room temperature for 24 hr. The crystallised product is filtered, washed with cold water and recrystallised from a suitable solvent. Results obtained are given in Table-7.

T A B L E - 7

N-Benzoylamino Acids prepared by hydrolysing their amides with concentrated hydrochloric acid (36%)

DL-N-Benzoylamino Acids	Heating time hr	% Yield Obtd.
DL-N-Benzoyl-β-1-naphthylalanine *	32	75
DL-N-Benzoyl-β-o-methoxyphenylalanine*	5	98
DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine	18	72
DL-N-Benzoylcyclohexylglycine*	12	93
DL-N-Benzoyl-o-tyrosine	24	70.5
DL-N-Benzoylnorleucine	10	75
DL-N-Benzoyl-ε;δ-dimethylisoleucine*	2	72.8
DL-N-Benzoyl-β;γ-dimethylleucine*	15	97
DL-N-Benzoyl-β-piperonylalanine	0.3	74

* Compounds reported for the first time

N-Benzoylamino acid amides containing amino or pyridyl groups in the molecules are hydrolysed by heating them with dilute hydrochloric acid (10%) for 0.2-0.6 hr and the compounds obtained after the removal of hydrochloric acid are neutralised carefully with dilute ammonia solution. Results are summarized in Table 8.

T A B L E -8

N-Benzoylamino acids prepared by Hydrolysing their amides with dilute hydrochloric acid (10%)

DL-N-Benzoylamino Acids	Heating time hr.	%Yield Obtd.
DL-N-Benzoyl-β-amino-β-(o-benzenecarboxylic acid)alanine*	0.5	68
DL-N-Benzoyl-β-p-dimethylamino-phenylalanine	0.3	54
DL-N-Benzoyl-β-m-aminophenyl-alanine*	0.6	60
DL-N-Benzoyl-ε-o-aminophenyl-norvaline*	0.5	96
DL-N-Benzoyl-δ;δ-dimethyl-δ-aminoisoleucine*	0.2	74
DL-N-Benzoyl-β-3-pyridylalanine	0.25	71

* Compounds reported for the first time

Hydrolysis of N-benzoyl-β-2;4-dihydroxyphenylalanine amide and N-benzoylcyclopentyglycine amide is more conveniently accomplished by warming them with sodium hydroxide solution (20%) on an electric hot plate till evolution of ammonia ceased. It is then acidified by dropwise addition of hydrochloric acid (36%) under cooled conditions and vigorous shaking. N-Benzoyl-β-2;4-dihydroxyphenylalanine (78%) and N-benzoylcyclopentylglycine (78%) are prepared in this manner.

N-Benzoylamino acid amides are purified by recrystallisation from aqueous ethanol (20-95%). N-Benzoyl-β-3-pyridylalanine, N-benzoyl-β-p-dimethylaminophenylalanine, N-Benzoylcyclopentylglycine are crystallised from methanol, benzene, n-hexane and petroleum ether respectively. Solvent mixture like benzene-carbon tetrachloride, n-hexane-chloroform and benzene-chloroform are also employed for re-crystallisation of N-benzoyl-β-m-aminophenylalanine, N-benzoyl-δ-o-aminophenylnorvaline and N-benzoyl-δ;δ-dimethyl-δ-aminoisoleucine respectively. N-Benzoylnorleucine is obtained in a well crystallised form from the reaction mixture and needs no further purification. Yields are generally very good.

SYNTHESIS OF AMINO ACIDS

Subjection of N-benzoylamino acid amides to hydrolysis at reflux temperature leads to simultaneous acission of amide and benzoyl groups and permits the subsequent isolation of amino acid salts. The hydrolysing agents used for this purpose are hydrochloric acid, hydriodic acid with red phosphorus and barium hydroxide solution.

N-Benzoylamino acid amides are refluxed with concentrated hydrochloric acid (36%) for varying lengths of time (9-18 hr). Benzoic acid separated is filtered and the filtrate is evaporated to dryness under reduced pressure. Amino acid salts thus obtained give amino acids on neutralisation. Results are given in Table-9.

T A B L E - 9

Amino Acids prepared by hydrolysing their N-Benzoylamino acid amides with concentrated hydrochloric acid (36%)

DL-Amino Acids	Reflux time, hr	% Yield obtd.
DL-β-o-Methoxyphenylalanine	13	56
DL-β-Amino-β-(o-benzenecarboxylic acid)alanine *	9	60
DL-Cyclopentylglycine	18	63
DL-Cyclohexylglycine	16	55
DL-o-Tyrosine	18	25
DL-Norleucine	5	80
DL-δ;δ-Dimethylisoleucine*	9	78
DL-β;γ-Dimethylleucine*	16	82

* Compounds reported for the first time

N-Benzoylamino acid amides containing amino or pyridyl groups in the molecules are hydrolysed with dilute hydrochloric acid (10%) at reflux temperature for 1 to 5 hr. The benzoic acid which separated is filtered off and the filtrate is evaporated to dryness. The amino acid salts obtained in this manner are neutralised to yield free amino acids. Results are shown in Table-10.

T A B L E -10

Amino Acids prepared by hydrolysing their N-benzoylamino acid amides with dilute hydrochloric acid (10%)

DL-Amino Acids	Reflux time hr	% Yield Obtd.
DL-β-p-Dimethylaminophenyl-alanine	5	71.5
DL-β-m-Aminophenylalanine	2	79
DL-δ-o-Aminophenylnorvaline*	2	74
DL-δ; δ-Dimethyl-δ-Amino-isoleucine*	1	62
DL-β-3-Pyridylalanine	1.5	95

* Compounds reported for the first time

N-Benzoylamino acid amides which are resistant to hydrochloric acid hydrolysis are refluxed with hydriodic acid (sp. gr. 1.7) containing red phosphorus for 1.5 to 2 hr. Unreacted phosphorus is filtered and the filtrate is dried under reduced pressure. The residue so obtained is dissolved in water and extracted several times with ether. The aqueous layer is neutralised to obtain amino acid. In some cases

yields are better than those obtained from hydrochloric acid hydrolysis. N-benzoyl-β-3-methoxy-4-hydroxyphenylalanine amide and N-benzoyl-β-o-methoxyphenylalanine amide on treatment with hydriodic acid and red phosphorus give β-3;4-dihydroxyphenylalanine and o-tyrosine. The methyl ether linkage is also broken under these hydrolysing conditions. Results obtained are shown in Table-11.

T A B L E - 11

Amino acids prepared by hydrolysing their N-benzoylamino acid amides with hydriodic acid and red phosphorus

DL-Amino Acids	Reflux time hr	% Yield Obtd.
DL-β-1-Naphthylalanine	2	75
DL-β-2;4-Dihydroxyphenylalanine	2	71
DL-β-3;4-Dihydroxyphenylalanine	2	90
DL-Cyclohexylglycine	2	64
DL-o-Tyrosine	2	89
DL-Norleucine	1.5	95

Stephens and Weizmann[79] demonstrated the conversion of piperonal to β-3;4-dihydroxyphenylalanine employing the Gabriel phthalimide procedure. Our attempts to hydrolyse N-benzoyl-β-piperonylalanine amide result in the formation of a black pigment under different acidic conditions and

no amino acid is isolated. However, hydrolysis of this amide at reflux temperature using barium hydroxide solution (15%) leads to the formation of β-piperonylalanine in 73.5% yield.

The amino acid salts obtained by acid hydrolysis are treated with silver oxide and the precipitated silver salt is removed from the reaction mixture by filtration. Traces of silver ions left in the solution are removed with water washed hydrogen sulphide gas. The amino acids isolated in this fashion are almost pure.

Amino acid salts of β-1-naphthylalanine, β-m-aminophenylalanine, β-p-dimethylaminophenylalanine and cyclohexylglycine are soluble in water but the free amino acids are insoluble. Some difficulties are encountered in the isolation of these amino acids when their salts are neutralised with silver oxide as the crystalline material thus precipitated adhere to the silver salts. Thus the solution of these amino acid salts are cautiously neutralised using dilute ammonia solution. The crystalline amino acids are filtered and then recrystallised using a suitable solvent to yield the pure compounds.

Most of the amino acids are crystallised from ethanol (40-95%). The crystallisation of β-1-naphthylalanine and β-o-methoxyphenylalanine are effected from boiling water. Ethanol-ethyl acetate mixture is employed for the crystallisation of β-o-aminophenylnorvaline and cyclopentylglycine. Cyclohexylglycine is recrystallised from glacial acetic acid.

E X P E R I M E N T A L

EXPERIMENTAL*

I. SYNTHESIS OF AZLACTONES

1. 2-Phenyl-4-(1'-naphthylmethylene)-5-oxazolone

A mixture of 1-naphthaldehyde, 15.6g (0.1 mol), hippuric acid, 17.9g (0.1 mol), freshly fused sodium acetate, 8.2g (0.1 mol) and acetic anhydride, 30 ml (0.3 mol) was fused on a free flame in a conical flask and then heated on a steam bath for 1 hr. After cooling, 30 ml of ethanol (95%) was added to the reaction mixture and then left overnight at room temperature. The crude product was filtered on a Buchner funnel, washed with three 50 ml portions of hot water and once with cold ethanol. It was recrystallised from ethanol (95%). Yellow, needle-shaped crystals thus obtained were filtered and dried. The yield was 18.8g (62.8%), mp 174-75° (lit[262] mp 170-71°).

2. 2-Phenyl-4-(o-methoxybenzal)-5-oxazolone

A mixture of o-methoxybenzaldehyde, 13.6g (0.1 mol), hippuric acid, 17.9g (0.1 mol), powdered freshly fused sodium acetate, 8.2g (0.1 mol), and acetic anhydride, 28.3 ml (0.3 mol) was heated on a steam bath for 30 min. Yellow crystals soon began to form and the whole liquid mass became solid. Ethanol (30 ml) was added slowly to decompose acetic anhydride. The crystalline material was filtered, washed with ethanol and then with hot water. It was recrystallised from ethanol (95%). The golden yellow

* Melting points reported in this work were taken on a Kofler hot block and are in degree centigrade.

needle shaped crystals so secured were dried and weighed 20.01g (75%), mp 166-67° (lit[263] mp 165-66°).

3. 2-Phenyl-4-(2',4'-diacetoxybenzal)-5-oxazolone

A mixture of β-resorcylaldehyde, 10.6g (0.1 mol), hippuric acid, 17.9g (0.1 mol), freshly fused sodium acetate, 8.2g (0.1 mol) and acetic anhydride, 28.3 ml (0.3 mol) was heated on a free flame. As soon as the mixture liquified completely, the flask was transferred to a steam bath and heated for 2 hr. At the end of this period, 25 ml of ethanol was added slowly while cooling the flask. After allowing the mixture to stand overnight, the crystalline product was filtered on a Buchner funnel, washed with two 20-ml portions of ice-cold ethanol and finally with two 20-ml portions of boiling water. On drying the product weighed 33.3g (91.2%) and melted at 136-37°. The crystallisation was effected from ethanol affording buff-coloured needles, mp 139-40° (lit[241] mp 240).

4. 2-Phenyl-4-(3'-methoxy-4'-acetoxybenzal)-5-oxazolone

An intimate mixture of vanillin, 15.2g (0.1 mol), hippuric acid, 17.9g (0.1 mol), and freshly fused powdered sodium acetate, 16.4g (0.2 mol) was heated with 28.3 ml (0.3 mol) of acetic anhydride on a steam bath for 15 min. The reaction mixture was then ground up with water, filtered and washed several times with water. The crude product was crystallised from glacial acetic acid to form yellow needles. The azlactone melted at 188-89° (lit[229] mp 188-89°) and weighed 22.1g (75%).

5. 2-Phenyl-4-phthalidene-5-oxazolone

Phthalic anhydride, 14.8g (0.1 mol), hippuric acid, 17.9g (0.1 mol), powdered anhydrous sodium acetate, 8.2g (0.1 mol) and acetic anhydride, 28.3 ml (0.3 mol) were taken in a conical flask. The contents were then heated on a low flame with constant shaking till a clear solution was obtained. The flask was then transferred to a steam bath and heated for 2 hr to ensure completion of the reaction, during this period the liquid turned to a brownish paste. The contents were then left overnight after adding 28 ml of ethanol. The precipitated oxazolone was filtered, washed with ice-cold ethanol and then with hot water. The dried product weighed 19 g (60.7%), mp 238-40°. Recrystallisation from ethanol raised the melting point to 249-50° (lit[183] mp 240°), IR (KBr) 1810 (5-member lactone C = O), 1765 (C = O), 1670 (C=N), 1620, 1550 Cm^{-1} (C = C, aromatic), 1300 (C - N)

6. 2-Phenyl-4-(p-dimethylaminobenzal)-5-oxazolone

A mixture of hippuric acid, 17.9g (0.1 mol), p-dimethylaminobenzaldehyde, 14.9 g(0.1 mol), finally powdered fused sodium acetate, 8.2g (0.1 mol) and acetic anhydride, 40.8 ml (0.4 mol) was refluxed for 20 min. The contents were poured into 100 ml of ice-cold water and the precipitated crystalline oxazolone was filtered, washed with plenty of water and once with ice-cold ethanol to remove unreacted aldehyde. After crystallisation from benzene, the azlactone was obtained as reddish brown

needles, mp 232-33 (lit[264] mp 210-11°), It weighed 13.5g (69.2%) IR (KBr) 1762 (azlactone C = O), 1650 (C = N), 1610 (C = C), 1530, 1380 Cm^{-1} (aromatic, C - N).

7. 2-Phenyl-4-cyclopentylidene-5-oxazolone

To a mixture of hippuric acid, 35.8g (0.2 mol), freshly fused sodium acetate, 8.2g (0.1 mol) and acetic anhydride, 56.6 ml (0.6 mol), cyclopentanone, 25.5g (0.3 mol) was added dropwise over a period of 4 hr at room temperature. After the addition was complete, the reaction mixture was refluxed for 30 min. The resulting clear, dark pink solution was then cooled to room temperature and finally added to 500 ml of cold water with vigorous stirring. The resulting semi solid material was extracted with five 80- ml portions of light petroleum ether (bp 40-60°). After evaporating the ether, the yellow product was crystallised from benzene yielding 7.5g (33%) of the oxazolone melting at 113-14°, Recrystallisation from ethanol (95%) raised the melting point of the product to 120-21° (lit[265] mp 116°).

8. 2-Phenyl-4-cyclohexylidene-5-oxazolone

A mixture of hippuric acid, 53.8g (0.3 mol), freshly fused sodium acetate, 24.6g (0.3 mol), acetic anhydride, 65.8 ml (0.7 mol) and cyclohexanone, 30g (0.3 mol) was heated on a steam bath for 45 min to yield a dark pink solution. The warm reaction mixture was then reduced to a

volume of 125 ml under reduced pressure when a portion of the oxazolone precipitated. The resulting residue was cooled for few hours, suspended in ethanol, and poured into 2 lit of ice-cold water with continuous stirring. The aqueous phase was decanted and the precipitate was dissolved in hot ethanol. On chilling, the oxazolone emerged in red needle shaped crystals. This was filtered and dried when it weighed 17.4g(24.6%), mp 140°. This was recrystallised from ethanol (95%) when the crystalline product obtained melted at 142° (lit[265] mp 142°).

9. 2-Phenyl-4-(o-acetoxybenzal)-5-oxazolone

A mixture of salicyladehyde 12.2g (0.1 mol), hippuric acid, 17.9g (0.1 mol), anhydrous potassium carbonate, 13.8g (0.1 mol) and acetic anhydride, 28.3 ml (0.3 mol) was stirred at room temperature. The temperature of the mixture was then raised gradually to about 100° when the mixture set into a yellow crystalline mass. It was allowed to stand overnight at room temperature, triturated with 100 ml of hot water and the granular material thus obtained was filtered, washed with three 20-ml portions of ethanol and dried when the product melted at 137-38° and weighed 21.8g(71%). Crystallisation from ethanol (95%) gave golden yellow crystals of the product, mp 138-39° (lit[266] mp 137-38°).

10. 2-Phenyl-4-(m-nitrobenzal)-5-oxazolone

To a mixture of m-nitrobenzaldehyde, 15.1g (0.1 mol), hippuric acid, 17.9g (0.1 mol) and anhydrous potassium carbonate, 13.8g (0.1 mol) was added 50 ml (0.6 mol) of acetic anhydride and the mixture was stirred

with a glass rod. Within 5 min the temperature of the mixture raised to 100°. The stirring was containued for further 15 min when a yellow crystalline product was obtained. This was left overnight at room temperature and then 50 ml of ethanol was added. The precipitated oxazolone was filtered, triturated with 25 ml of ice-cold ethanol and with hot water several times. This was recrystallised from ethanol (95%) to afford yellow crystals weighing 29.4g and melting at 175-76° (lit[267] mp 174°). The yield was nearly quantitative. IR(KBr) 1760 (azlactone C = O), 1650, 1610 (C = N, C = C), 1520, 1320 Cm^{-1} (aromatic, C - N).

11. 2-Phenyl-4-(o-nitrocinnamylidene)-5-oxazolone

o-Nitrocinnamaldehyde, 8.85g (0.05 mol), hippuric acid, 8.95g (0.05 mol), acetic anhydride, 14.1 ml (0.51 mol) and anhydrous potassium carbonate, 6.9g (0.05 mol) were stirred together at room temperature. After few minutes the mixture was liquified and the temperature raised to 60°. The stirring was containued till the mixture set into a yellow crystalline mass. This was allowed to stand overnight at room temperature. Then 200 ml of hot water was added, the precipitated oxazolone was filtered and washed with ethanol. The dried product melted at 197-98° and weighed 14.8g. The yield of the product was almost quantitative. Crystallisation from ethyl acetate raised the melting point to 217-18° (lit[268] mp 201°).

12. 2-Phenyl-4-crotonylidene-5-oxazolone

To a mixture of hippuric acid, 17.9 (0.1 mol), anhydrous potassium carbonate, 13.9g (0.1 mol) and acetic anhydride, 28.3 ml (0.3 mol),

7.7g (0.11 mol) of crotonaldehyde was added dropwise with constant stirring. The reaction mixture was liquified to dark pink colour within 5 min and the temperature raised upto 100°. The stirring was continued for 20 min till it was solidified and then left overnight at room temperature. Acetic anhydride was decomposed by adding water and the precipitated oxazolone was filtered. This was washed with three 50 ml portions of hot water and once with ice-cold ethanol. On drying the product melted at 156-57° and weighed 8.5g (40%). Recrystallisation from ethanol yielded pink, needle-shaped crystals, mp 163-64°.

Anal. for $C_{13}H_{11}O_2N$, Calcd: C, 73.22; H, 5.20; N, 6.57
Found: C, 72.91; H, 5.43; N, 6.37

13. 2-Phenyl-4-(1',3'-dimethyl-3'-aminobutylidene)-5-oxazolone

A mixture of diacetoneamine, 23.46g (0.24 mol), hippuric acid, 35.8g (0.2 mol), acetic anhydride, 51 ml (0.5 mol) and anhydrous lead acetate, 32.5g (0.1 mol) was heated under reflux for 25 min and the clear orange solution obtained was poured into 500 ml of water. After few hours a gummy product was separated and this was extracted with five 80- ml portions of boiling light petroleum ether (bp 40-60°). The petroleum ether extract on evaporation gave a yellow solid (18.2g, 36.2%) which melted at 116-17°. Recrystallisation from benzene-hexane mixture (1:4) gave light yellow crystals, mp 120-21°.

Anal. for $C_{15}H_{18}O_2N_2$, Calcd: C, 69.76; H, 7.02; N, 10.85
Found: C, 69.39; H, 7.29; N, 11.13.

14. 2-Phenyl-4-(1';3'-dimethyl-2'-butenylidene)-5-oxazolone

A mixture of mesityl oxide, 22.6g (0.22 mol), hippuric acid, 35.8g (0.2 mol), acetic anhydride, 56.6 ml (0.6 mol) and anhydrous lead acetate, 32.5g (0.1 mol) was refluxed for 25 min and the clear brown solution thus obtained was poured into 600 ml of water. After some hours the gummy product was separated and this was extracted with five 80-ml portions of boiling light petroleum ether (bp 40-60°). The petroleum ether extract on evaporation gave 16.4g (34%) of a light yellow solid, mp 121-22°. Crystallisation from chloroform-hexane mixture (1:3) raised the melting point to 129-30°.

Anal for $C_{15} H_{15} O_2 N$, Calcd: C, 74.66; H, 6.27; N, 5.80

Found C, 74.37; H, 6.35; N, 5.98

15. 2-Phenyl-4-(1';2';2'-trimethylpropylidene)-5-oxazolone

A mixture of hippuric acid, 35.8g (0.2 mol), pinnacolone, 24g (0.24 mol), acetic anhydride, 56.6 ml (0.6 mol) and anhydrous lead acetate, 32.5g (0.1 mol) was heated under reflux for 25 min and the clear orange solution thus obtained was poured into 600 ml of water. After some hours the gummy product was extracted with five 80-ml portions of boiling light petroleum ether (bp 40-60°). The petroleum ether extract on evaporation gave 20.4g (42%) of pink crystalline product, mp 112-13°. Recrystallisation from carbontetrachloride raised the melting point to 119-20°.

Anal for $C_{15} H_{17} O_2 N$, Calcd: C, 74.05; H, 7.04; N, 5.76

Found: C, 74.38; H, 6.76; N, 6.03.

16. 2-Phenyl-4-(3'-pyridylmethylene)-5-oxazolone

Hippuric acid, 18g (0.1 mol) and potassium bicarbonate, 4g (0.04 mol) were dissolved in acetic anhydride, 40 ml (0.4 mol) with stirring. The reaction vessel was cooled by a water bath to maintain a temperature near 20°. Pyridyl-3-aldehyde, 10 ml (0.114 mol) was then added atonce and the mixture was stirred for 1 hr. The semisolid tan mixture was then poured into 200 ml of hot distilled water. The precipitated oxazolone thus obtained was filtered, washed with water and dried. The yield was 23.1 g (92.4%), mp 156-57. After being triturated with ethanol, the redried product melted at 163-64° (lit[257] mp 164-65°).

17. 2-Phenyl-4-piperonalmethylene-5-oxazolone

Piperonal, 15g (0.1 mol), hippuric acid, 17.9g (0.1 mol), acetic anhydride, 24.4 ml (0.2 mol) and potassium bicarbonate, 10g (0.1 mol) were stirred together at room temperature. The internal temperature gradually raised to about 100° and the mixture set into a crystalline mass. It was allowed to stand overnight at room temperature and treated with 150 ml of hot water. The crude oxazolone was filtered and washed with dilute acetic acid followed by water. The dry material weighed 20g (82%). On recrystallisation from ethanol (95%), it melted at 197-98° (lit[186] mp 195-97°).

HYDROGENATION OF AZLACTONES*

II. PREPARATION OF N-BENZOYLAMINO ACID AMIDES

RANEY NICKEL CATALYSED REDUCTION

1. DL-N-Benzoyl-β-1-naphthylalanine amide

Powdered 2-phenyl-4-(1'-naphthylmethylene)-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this 3.5g of freshly prepared Raney nickel catalyst was added alongwith 10 ml of concentrated ammonia solution (sp. gr. 0.9). This was reduced under a hydrogen pressure of 47 psi in a Paar catalytic hydrogenation apparatus for 10 hr. The flask was disconnected and contents were heated on a steam bath to dissolve the white precipitated benzoylamino acid amide and filtered hot. The catalyst was washed with three 20-ml portions of boiling ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (95%). The crystalline material was filtered and dried, yield 5.73g (90%). It melted at 221-22°.

Anal for $C_{20}H_{18}O_2N_2$, Calcd: C, 75.47; H, 5.70; N, 8.80,
Found: C, 75.75; H, 5.91, N, 8.52.

2. DL-N-Benzoyl-β-o-methoxyphenylalanine Amide

Powdered 2-phenyl-4-(o-methoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this freshly prepared Raney nickel catalyst (3g) was added alongwith 9 ml of concentrated ammonia. This was reduced

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* All reductions were conducted in a Paar catalytic hydrogenation apparatus.

under a hydrogen pressure of 45.8 psi. The reduction was complete in 11 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (95%). The crystalline material thus obtained was filtered and dried when it weighed 3.91g (61%) and melted at 220-21°.

Anal for $C_{17}H_{18}O_3N_2$, Calcd: C, 68.44; H, 6.08; N, 9.40

Found: C, 68.79; H, 6.05; N, 9.59

3. DL-N-Benzoyl-β-2,4-dihydroxyphenylalanine Amide

Powdered 2-phenyl-4-(2,4-diacetoxy-benzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml) and Raney nickel catalyst (3g). This was reduced under a hydrogen pressure of 42 psi. The reduction was complete in 7 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (95%). The crystalline product was filtered and dried, yield 3.79g (78%), mp 286-87°.

Anal for $C_{16}H_{16}O_4N_2$, Calcd: C, 64.0; H, 5.37; N, 9.33,

Found: C, 63.81; H, 5.34; N, 9.42.

4. DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine Amide

Powdered 2-phenyl-4-(3'-methoxy-4-acetoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml), and Raney nickel catalyst (3g). This was reduced under a hydrogen pressure of 43 psi. After 10 hr when there was no more obsorption of hydrogen, the flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was concentrated to 25 ml under reduced pressure when the amide crystallised on cooling. This was filtered and dried when it melted at 209-10° (lit[256] mp 209-10°) and weighed 4.47g (80%).

Anal for $C_{17}H_{18}O_4N_2$, Calcd: C, 64.96; H, 5.77; N, 8.91

Found C, 65.29; H, 5.46; N, 8.58.

5. DL-N-Benzoyl-β-amino-β-(o-benzenecarbonamide)alanine Amide

Powdered 2-phenyl-4-phthalidene-5-oxazolone (5g) was suspended in 100 ml of ethanol followed by the addition of ammonia solution (10 ml) and freshly prepared Raney nickel catalyst (4g). This was reduced under a hydrogen pressure of 52 psi. The reduction was complete in 10.4 hr. The flask was disconnected, contents heated on a steam bath and filtered hot followed by washing of the catalyst with boiling ethanol. The combined filtrate and washings were evaporated under reduced pressure. The residue so obtained was crystallised from ethanol (95%). The crystalline amide

was filtered and dried when it weighed 3.3g (95%) and melted at 196-97°. IR(KBr) 3370-3150 (NH_2, N-H), 1670-1650 (amide C = O), 1630, 1540 (N-H, aromatic C = C), 1330 and 1250 Cm^{-1} (aromatic and aliphatic C - N).

Anal for $C_{17}H_{18}O_3N_4$, Calcd: C, 62.57; H, 5.56; N, 17.17

Found: C, 62.25; H, 5.79; N, 16.84.

6. DL-N-Benzoyl-β-p-dimethylaminophenylalanine Amide

Powdered 2-phenyl-4-(p-dimethylaminobenzal)-5-oxazolone (5.5g) was suspended in 100 ml of ethanol. To this freshly prepared Raney nickel catalyst (3g) was added alongwith 9 ml of ammonia solution. This was reduced under a hydrogen pressure of 49.5 psi. The reduction was complete in 6 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was concentrated to 30 ml under reduced pressure when the amide was crystallised on keeping the solution. The crystalline product was filtered and dried when it weighed 2g and melted at 251-52°. As the amide was sparingly soluble in hot ethanol therefore the catalyst was washed on a Buchner funnel thtice with 10-ml portions of warm glacial acetic acid and the washings were left for crystallisation overnight. The crystalline material thus separated was filtered, washed withethanol and dried. It weighed 1.8g and melted at 251-52°. Total yield of the amide was 3.8g (65%). IR(KBr) 3337 (NH_2), 3280 (N - H), 1650 (primary amide C = O), 1630 (sec. amide C = O), 1520, 1330 CM^{-1} (p-subtd. phenyl, C-N).

Anal for $C_{18}H_{21}O_2N_3$, Calcd: C, 69.43; H, 6.80; N, 13.50

Found: C, 69.68; H, 6.61; N, 13.43.

7. DL-N-Benzoylcyclopentylglycine Amide

Powdered 2-phenyl-4-cyclopentylidene-5-oxazolone (5g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml) and Raney nickel catalyst (3.2g). This was reduced under a hydrogen pressure of 50 psi. The reduction was complete in 9 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue so obtained was crystallised from benzene-ethanol mixture (1:4). The crystalline amide was filtered and dried when it weighed 3.4g (63%) and melted at 190-91°.

Anal for $C_{14} H_{18} O_2 N_2$: Calcd: C, 68.27; H, 7.37; N, 11.37
Found: C, 67.91; H, 7.68; N, 11.51.

8. DL-N-Benzoylcyclohexylglycine Amide

Powdered 2-phenyl-4-cyclohexylidene-5-oxazolone (5g) was suspended in 100 ml of ethanol, Raney nickel catalyst (2.5g) was added alongwith 10 ml of concentrated ammonia solution and it was reduced under a hydrogen pressure of 49 psi. After 5 hr the reduction was complete. The flask was disconnected contents heated on a steam bath and filtered hot. The filtrate was concentrated to 50 ml when the amide was crystallised on keeping. The crystalline product was filtered and dried when it melted at 269-70° and weighed 3.64g. Mother liquor on further concentration gave another 0.72g of the product. The total yield was 4.36g (81%).

Anal for $C_{15} H_{20} O_2 N_2$, Calcd : C, 69.20; H, 7.74; N, 10.76
Found : C, 68.89; H, 7.48; N, 10.83.

9. DL-N-Benzoyl-o-tyrosine Amide

Powdered 2-phenyl-4-(o-acetoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol alongwith 10 ml of ammonia solution and Raney nickel catalyst (3g). This was reduced under a hydrogen pressure of 58 psi. The reduction was complete in 5.5 hr. The flask was disconnected, contents heated on a steam bath and filtered hot followed by washing the catalyst with boiling ethanol. The combined filtrate and washings were dried under reduced pressure and the residue was crystallised from ethanol (95%). The amide thus obtained on drying melted at 177-78° and weighed 4.92g (82%).

Anal for $C_{16}H_{16}O_3N_2$, Calcd: C, 67.60; H, 5.67; N, 9.85
Found: C, 67.39; H, 5.43; N, 10.12.

10. DL-N-Benzoylnorleucine Amide

Powdered 2-phenyl-4-crotonylidene-5-oxazolone (5.5g) was suspended in 100 ml of ethanol containing ammonia solution (8.5 ml) and Raney nickel catalyst (3g). This was reduced under a hydrogen pressure of 39 psi. The reduction was completed in 3 hrs. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was dried under reduced pressure and the residue was crystallised from aqueous ethanol (30%). The crystalline amide thus obtained on drying weighed 3.74g (62%) and melted at 161-62° (lit[273] mp 143-44°).

Anal for $C_{13}H_{18}O_2N_2$, Calcd: C, 66.66; H, 7.74; N, 11.96
Found: C, 66.81; H, 7.49; N, 11.93

11. DL-N-Benzoyl- δ; δ -dimethyl-δ -aminoisoleucine Amide

Powdered 2-phenyl-4-(1',3'-dimethyl-3'-aminobutylidene)-5-oxazolone (6.5g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml) and Raney nickel catalyst (4g). This was reduced under a hydrogen pressure of 46 psi. The reduction was complete in 5 hr. The contents were boiled on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethyl acetate. The amide so obtained on drying melted at 145-46° and weighed 3.84g (55%).

Anal for $C_{15} H_{23} O_2 N_3$, Calcd: C, 64.94; H, 8.36; N, 15.16.

Found: C, 64.89; H, 8.12; N, 15.33.

12. DL-N-Benzoyl- δ; δ -dimethylisoleucine Amide

Powdered 2-phenyl-4-(1'-3'-dimethyl-2'-butenylidene)-5-oxazolone (5g) was suspended in 100 ml of ethanol, Raney nickel catalyst (2.5g) was added alongwith 9 ml of a mmonia solution ans this was reduced under a hydrogen pressure of 53 psi. After 8 hr the reduction was complete. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue so secured was crystallised from benzene-methanol mixture (1:3). The crystalline amide thus obtained was filtered and dried when it melted

at 196-97° and weighed 3.35g (58%).

Anal for $C_{15}H_{22}O_2N_2$, Calcd: C, 68.67; H, 8.45; N, 10.68

Found: C, 68.43; N, 8.21; N, 10.99

13. DL-N-Benzoyl-β,γ-dimethylleucine Amide

Powdered 2-phenyl-4-(1'-2',3'-trimethylpropylidene)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (9 ml) and freshly prepared Raney nickel catalyst (3.5g). This was reduced under a hydrogen pressure of 38 psi for 6 hr. When there was no more absorption, the flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethyl acetate-chloroform mixture (3:2). The amide thus obtained on drying weighed 4g (62.4%) and melted at 166-67°.

Anal for $C_{15}H_{22}O_2N_2$, Calcd: C, 68.67; H, 8.47; N, 10.68

Found: C, 69.11; H, 8.32; N, 10.35.

14. DL-N-Benzoyl-β-piperonylalanine Amide

Powdered 2-phenyl-4-piperonalmethylene-5-oxazolone (5g) was suspended in 100 ml of ethanol alongwith Raney nickel catalyst (2.5g) and ammonia solution (8 ml). This was reduced under a hydrogen pressure of 42.8 psi. After 8 hr the reduction was complete. The flask was disconnected, contents heated on a steam-bath and filtered hot. The filtrate was evaporated to dryness and the residue was crystallised from ethanol (95%). The

crystalline amide so obtained melted at 215-16° and weighed 3.78g. Mother liquor on concentration yielded another 0.5g of the product. The total yield was 4.48g (82.5%).

Anal for $C_{17}H_{16}O_4N_2$, Calcd: C, 65.37; H, 5.16; N, 8.97

Found: C, 65.19; H, 5.38; N, 8.56.

PALLADIUM-CHARCOAL (10% Pd) CATALYSED REDUCTION

1. DL-N-Benzoyl-β-1-naphthylalanine Amide

Powdered 2-phenyl-4-(1'-naphthylmethylene)-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this palladium charcoal (0.5g) was added alongwith 9 ml of concentrated ammonia solution. This was reduced under a hydrogen pressure of 36 psi in a Paar catalytic hydrogenation apparatus for 8 hr. The flask was disconnected, contents heated on a steam bath to dissolve the white precipitated benzoylamino acid amide and filtered hot. The catalyst was washed with three 20-ml portions of boiling ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (95%). The crystalline material thus obtained was filtered and dried, yield 6.05g (95%), mp 221-22°; mixed mp 222°.

2. DL-N-Benzoyl-β-o-methoxyphenylalanine Amide

Powdered 2-phenyl-4-(o-methoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (9 ml) and Palladium charcoal (0.5g). This was reduced under a hydrogen pressure of 41 psi for 9 hr. When there was no more absorption of hydrogen, the flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (95%). The crystalline product thus obtained was filtered and dried when it weighed 4.96g (77.4%) and melted

at 220-21°. No depression was observed, in a mixed melting point determination with an authentic sample.

3. DL-N-Benzoyl-β-2;4-dihydroxyphenylalanine Amide

Powdered 2-phenyl-4-(2;4-diacetoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml) and palladium charcoal (0.5g). This was reduced under a hydrogen pressure of 38 psi. After 5 hr when there was no more absorption of hydrogen, the flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (95%). The amide thus obtained melted at 286-87° and weighed 3.79 (78%). Mixed mp with an authentic sample showed no depression.

4. DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine Amide

Powdered 2-phenyl-4-(3'-methoxy-4-'-acetoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml) and palladium charcoal (0.5g). This was reduced under a hydrogen pressure of 39.2 psi for 6.5 hr. After the completion of reduction the flask was disconnected, contents heated on a steam bath and filterated hot. The filtrate was dried under reduced pressure and the residue was crystallised from ethanol (95%). The amide so obtained melted at 209-10° and weighed 4.75g (85%). Mixed mp with an authentic sample was not depressed.

5. DL-N-Benzoyl-β-amino-β-(o-benzenecarbonamido) alanine Amide

Powdered 2-phenyl-4-phthalidene-5-oxazolone (5g) was suspended in 100 ml of ethanol followed by the addition of ammonia solution (10 ml) and palladium charcoal (0.45g). This was reduced under a hydrogen pressure of 43 psi. The reduction was complete in 8 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The catalyst was washed with three 30 ml portions of boiling ethanol. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue was crystallised from ethanol (95%). The crystalline amide thus obtained on drying weighed 3.7g (64.5%) and melted at 196-97°. No depression in a mixed mp determination with an authentic sample was observed.

6. DL-N-Benzoyl-β-p-dimethylaminophenylalanine Amide

Powdered 2-phenyl-4-(-dimethylaminobenzal)-5-oxazolone (5.5g) was suspended in 100 ml of ethanol. To this palladium charcoal (0.5g) was added alongwith 9 ml of ammonia solution. This was reduced under a hydrogen pressure of 40 psi for 4 hr. When there was no more reduction, the flask was disconnected, contents boiled on a steam bath and filtered hot. The catalyst was washed on the Buchner funnel thrice with 10-ml portions of warm glacial acetic acid. The filtrate and washings were evaporated to dryness under reduced pressure. The residue thus obtained was dissolved in minimum amount of glacial acetic acid and left for crystallisation. The crystalline product was filtered, washed with two 10 ml

portions of ethanol and dried when it weighed 4.16g (71%) and melted at 251-52°. There was no depression in a mixed mp determination with an authentic sample.

7. DL-N-Benzoylcyclopentylglycine Amide

Powdered 2-phenyl-4-cyclopentylidene-5-oxazolone (5g) was suspended in 100 ml of ethanol containing ammonia solution (8.5 ml) and palladium charcoal (0.4g). This was reduced under a hydrogen pressure of 45 psi for 8 hr. After this period, the flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from benzene-ethanol mixture (1:4). The amide thus obtained was filtered and dried when it weighed 3.62g (67%) and melted at 190-91°. Mixed mp with an authentic sample of the amide obtained using Raney nickel datalyst was not depressed.

8. DL-N-Benzoylcyclohexylglycine Amide

Powdered 2-phenyl-4-cyclohexylidene-5-oxazolone (5g) was suspended in 100 ml of ethanol. To this palladium charcoal (0.4g) and 10 ml of ammonia solution was added and this was reduced under a hydrogen pressure of 38 psi. The reduction was complete in 5 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was dried under reduced pressure and the residue was cyystallised

from ethanol (95%). The amide so obtained melted at 269-70° and weighed 4.64g (86%). Mixed mp with an authentic sample showed no depression.

9. DL-N-Benzoyl-o-tyrosine Amide

Powdered 2-phenyl-4-(o-acetoxybenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this ammonia solution (10 ml) and palladium charcoal (0.5g) was added. This was reduced under a hydrogen pressure of 49.6 psi. The reduction was complete in 4 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was dried under reduced pressure and the residue was crystallised from ethanol (95%). The amide thus obtained on drying melted at 177-78° and weighed 5.16g (86%). Mixed mp with an authentic sample was not depressed.

10. DL-N-Benzoyl-β-m-aminophenylalanine Amide

Powdered 2-phenyl-4-(m-nitrobenzal)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml) and palladium charcoal (0.5g). This was reduced under a hydrogen pressure of 50 psi for 2.5 hr. When there was no more absorption of hydrogen, the flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was dried under reduced pressure and the residue was crystallised from ethanol (95%). The crystalline amide thus obtained melted at 209-10° and weighed 5.6g (86%). IR (KBr) 3220 (NH_2), 1660, 1615, 1530 (C = O, aromatic, C = C), 1310 Cm^{-1} (aromatic C - N).

Anal for $C_{16}H_{17}O_2N_3$, Calcd: C, 67.82; H, 6.05; N, 14.84
Found: C, 67.53; H, 6.32; N, 14.46.

11. DL-N-Benzoyl-δ-o-aminophenylnorvaline Amide

Powdered 2-phenyl-4-(o-nitrocinnamylidene)-5-oxazolone (6.5g) was suspended in 100 ml of ethanol containing ammonia solution (10 ml) and palladium charcoal (0.5g). This was reduced under a hydrogen pressure of 47 psi. The reduction was complete in 1 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethyl acetate. The crystalline amide thus obtained on drying weighed 6.26g (nearly quantitative yield) and melted at 150-51°.

Anal for $C_{18}H_{21}O_2N_3$, Calcd: C, 69.43; H, 6.80, N, 13.50
Found: C, 69.72; H, 6.69; N, 13.50

12. DL-N-Benzoylnorleucine Amide

Powdered 2-phenyl-4-crotonylidene-5-oxazolone (5.5g) was suspended in 100 ml of ethanol containing ammonia solution (8 ml) and palladium charcoal (0.4g). This was reduced under a hydrogen pressure of 28 psi. The reduction was complete in 3 hr. The flask was disconnected, contents heated on a steam bath and filtered hot. The filtrate was dried under reduced pressure and the residue was crystallised from aqueous ethanol (30%). The crystalline amide on drying weighed 3.98g (68%) and melted at 161-62°. No depression in a mixed mp determination with an authentic sample was observed.

13. DL-N-Benzoyl-$\delta;\delta$-dimethyl-δ-aminoisoleucine Amide

Powdered 2-phenyl-4-(1',3'-dimethyl-3'-aminobutylidene)-5-oxazolone (6.5g) was suspended in 100 ml of ethanol containing ammonia solution (9-ml) and palladium charcoal (0.45g). This was reduced under a hydrogen pressure of 42 psi. The reduction was complete in 5 hr. The flask was disconnected, contents boiled on a steam bath and filtered. The filtrate was dried under reduced pressure and the residue was crystallised from ethyl acetate. The amide obtained in this manner on drying melted at 145-46° and weighed 4.8g (69%). Mixed mp with an authentic sample was not depressed.

14. DL-N-Benzoyl-$\delta;\delta$-dimethylisoleucine Amide

Powdered 2-phenyl-4-(1',3'-dimethyl-2'-butenylidene)-5-oxazolone (5g) was suspended in 100 ml of ethanol, palladium-charcoal (0.4g) was added alongwith 9 ml of ammonia solution and this was reduced under a hydrogen pressure of 39 psi. After 5.2 hr the reduction was complete and the flask was disconnected. The contents were heated on a steam bath and filtered hot. The filtrate was dried under reduced pressure and the residue was crystallised from benzene-methanol mixture (1:3). The amide thus obtained weighed 3.7g (68%), melted at 196-97° and showed no depression in a mixed mp determination with an authentic sample.

15. DL-N-Benzoyl-β,γ-dimethylleucine Amide

Powdered 2-phenyl-4-(1',2',2'-trimethylpropylidene)-5-oxazolone (6g) was suspended in 100 ml of ethanol containing ammonia solution (9 ml) and palladium charcoal (0.4g). This was reduced under a hydrogen pressure of 32 psi for 5 hr. When there was no more absorption of hydrogen, the flask was disconnected, contents heated on a steam bath and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was crystallised from ethyl acetatechloroform mixture (3:2). The amide thus obtained melted at 166-67° and weighed 4.2g (65%). Mixed mp with an authentic sample was not depressed.

16. DL-N-Benzoyl-β-3-pyridylalanine Amide

Powdered 2-phenyl-4-(3'-pyridylmethylene)-5-oxazolone (6g) was suspended in 100 ml of ethanol. To this palladium charcoal (0.5g) was added alongwith 10 ml of ammonia. This was reduced under a hydrogen pressure of 36 psi. The reduction was complete in 7 hr. The flask was disconnected, contents boiled on a steam bath and filtered. The filtrate was dried under reduced pressure and the residue was crystallised from ethanol-ethyl acetate mixture (1:4). The amide thus obtained weighed 6.4g (nearly quantitative yield) and melted at 189-90°.

Anal for $C_{15}H_{15}O_2N_3$, Calcd: C, 66.91; H, 5.61; N, 15.61

Found: C, 66.53; H, 5.81; N, 15.92.

17. DL-N-Benzoyl-β-piperonylalanine Amide

Powdered 2-phenyl-4-piperonalmethylene-5-oxazolone (5g) was suspended in 100 ml of ethanol alongwith palladium charcoal (0.4g) and ammonia solution (8 ml). This was reduced under a hydrogen pressure of 40 psi for 5 hr. When there was no more absorption of hydrogen, the flask was disconnected, contents boiled on a steam bath and filtered. The filtrate was dried under reduced pressure, and the pesidue was crystallised from ethanol (95%). The amide thus obtained melted at 215-16° and weighed 4.52g (85%). Mixed mp with an authentic sample was not depressed.

HYDROLYSIS OF N-BENZOYLAMINOACID AMIDES

III. PREPARATION OF N-BENZOYLAMINO ACID

1. DL-N-Benzoyl-β-1-naphthylalanine

DL-N-Benzoyl-β-1-naphthylalanine amide (2g) was taken alongwith 40 ml of concentrated hydrochloric acid (36%, A.R.) in a round bottomed flask fitted with a double layer reflux condenser and this was heated on an electric hot plate for 32 hr to obtain a clear solution. The flask was left overnight at room temperature. The white needle-shaped crystalline product thus separated was filtered, washed with water and dried. It weighed 1.1g and melted at 244-45°. On diluting and cooling the filtrate, a second crop of crystals (0.36g) was obtained. The total yield was 1.46g (75%). On recrystallisation from aqueous alcohol (60%) the benzoylamino acid melted at 245-46°.

Anal for $C_{20}H_{17}O_3N$, Calcd: C, 75.23; H, 5.37; N, 4.39
Found: C, 74.92; H, 5.01; N, 4.63.

2. DL-N-Benzoyl-β-o-methoxyphenylalanine

DL-N-Benzoyl-β-o-methoxyphenyl-alanine amide (2g) was taken in a flask containing 40 ml of concentrated hydrochloric acid. This was heated on a steam bath for 1 hr and then left at room temperature for 4 hr. After this period it was diluted with 30 ml of water and again allowed to stand overnight at room temperature whereupon crystalline benzoylamino acid thus obtained was filtered, washed with 30 ml of water and dried, mp 168-69°, yield 1.8g. A second crop of crystals (0.19g) was obtained on concentrating

the mother liquor under reduced pressure to about 25 ml. The total yield of the product was 1.99g (nearly quantitative). No change in mp was observed after recrystallisation from aqueous ethanol (50%).

Anal for $C_{17}H_{17}O_4N$, Calcd: C, 68.21; H, 5.73; N, 4.68
Found: C, 68.49; H, 5.91; N, 4.75.

3. DL-N-Benzoyl-β-2;4-dihydroxyphenylalanine

DL-N-Benzoyl-β-2;4-dihydroxyphenylalanine amide (2g) was warmed with 15 ml of 20% sodium hydroxide solution on an electric hot plate. The heating was stopped when the evolution of ammonia ceased. The contents were acidified to Congo red by dropwise addition of concentrated hydrochloric acid with vigorous shaking and thorough cooling. Benzoylamino acid was crystallised out on cooling in a refrigerator. The crystalline material was filtered, washed several times with water and dried. The drude benzoylamino acid thus obtained weighed 1.56g (78%) and melted at 225-26°. Recrystallisation from 30% ethanol raised the melting point to 228-26°.

Anal for $C_{16}H_{15}O_5N$, Calcd: C, 63.78; H, 5.02; N, 4.65
Found: C, 64.13; H, 5.27; N, 4.43.

4. DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine

DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid. The mixture was warmed

on an electric hot plate till a clear solution was obtained and then left at room temperature for 15 hr. The crystalline benzoylamino acid was filtered, washed with three 10-ml portions of water and dried. It melted at 160-61° and weighed 1.5g (72%). Recrystallisation from ethanol raised the melting point to 162-63° (lit[274] mp 164°). Mixed melting point with an authentic sample showed no depression.

5. DL-N-Benzoyl-β-amino-β-(o-benzenecarboxylic acid) Alanine

DL-N-Benzoyl-β-amino-β-(o-benzenecarbonamido) alanine amide 92g) was suspended in 20 ml dilute hydrochloric acid (10%) and heated gently on a steam bath for 30 min. The mixture was evaporated to dryness under reduced pressure and the residue was dissolved in 25 ml of water. This was neutralised carefully with dilute ammonia solution when the benzoylamino acid was crystallised out on cooling. The yellow, long needle shaped crystals were filtered, washed with cold water and dried. It weighed 1.1g and melted at 271-72°. The mother liquor on concentration yielded another 0.26g of the product. The total yield was 1.36g (68%). Recrystallisation from 25% ethanol raised the melting point to 286-87°.

Anal for $C_{17}H_{16}O_5N_2$, Calcd: C, 62.19; H, 4.91; N, 8.53

Found: C, 62.42; H, 4.58; N, 8.69.

6. DL-N-Benzoyl-β-p-dimethylaminophenylalanine

DL-N-Benzoyl-β-p-dimethylaminophenylalanine amide (2g) was suspended in dilute hydrochloric acid (20 ml) and warmed gently on a steam bath for 20 min. The contents were cooled, neutralised with ammonia and 20 ml of ethanol was added. On cooling white, needle shaped product was crystallised out . It was filtered, washed with ice-cold alcohol and dried. It melted at 137-38° and weighed 1g (54%). Recrystallisation from benzene raised the melting point to 145-46° (lit[278] mp 128-32°).

Anal for $C_{18}H_{20}O_3N_2$, Calcd: C, 69.21; H, 6.45; N, 8.97

Found: C, 69.39, H, 6.19; N, 9.23

7. DL-N-Benzoylcyclopentylglycine

DL-N-Benzoylcyclopentylglycine amide (2g) was warmed on an electric hot plate with 20 ml of 20% sodium hydroxide solution. The heating was stopped when the evolution of ammonia ceased. The contents were acidified to Congo red by dropwise addition of concentrated hydrochloric acid with vigorous shaking and thorough cooling. The product crystallised out on cooling was filtered, washed several times with cold water and dried. The melting point of N-Benzoylamino acid was 108-9°. On recrystallisation from petroleum ether (bp 60-80°) the mp raised to 110-11°. The yield of the product was 1.86g (93%).

Anal for $C_{14}H_{17}O_3N$, Calcd: C, 67.99; H, 6.93; N, 5.66

Found: C, 67.84; H, 6.98; N, 5.72

8. DL-N-Benzoylcyclohexylglycine

DL-N-Benzoylcyclohexylglycine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid. This was warmed on a steam bath for 12 hr with constant shaking and then cooled to room temperature. The white precipitated product was filtered, washed with three 15-ml portions of water and dried at 90° in an oven. It melted at 204-5° and weighed 1.86g(93%). Recrystallisation from ethanol (95%) raised the melting point to 216-17°.

Anal for $C_{15}H_{19}O_3N$, Calcd: C, 68.94; H, 7.33; N, 5.36

Found: C, 69.21; H, 7.43; N, 4.66.

9. DL-N-Benzoyl-o-tyrosine

Finely powdered DL-N-Benzoyl-o-tyrosine amide (3g) was suspended in 40 ml of concentrated hydrochloric acid in a round bottomed flask fitted with a reflux condenser. This was warmed on an electric hot plate for 24 hr. The contents were diluted with 25 ml of water and left at room temperature overnight. The crystalline benzoyl amino acid was filtered, washed with two 10-ml portions of water and dried in an oven at 90°. Recrystallisation from ethanol (95%) raised the mp to 174-75° (lit[275] mp 176°).

Anal for $C_{16}H_{15}O_4N$, Calcd: C, 67.36; H, 5.30; N, 4.91

Found: C, 67.57; H, 5.39; N, 4.73.

10. DL-N-Benzoyl-β-m-aminophenylalanine

DL-N-Benzoyl-β-m-aminophenylalanine amide (2g) was suspended in 20 ml of dilute hydrochloric acid and warmed gently on a steam bath for 35 min. The acid was evaporated to dryness under reduced pressure, residue dissolved in 20 ml of water and neutralised carefully with dilute ammonia. The resulting solution was dried under reduced pressure and the residue was crystallised from benzene-carbontetrachloride mixture (3:2). The N-benzoylamino acid thus obtained melted at 180-81° and weighed 1.2g(60%).

Anal for $C_{16}H_{16}O_3N_2$, Calcd: C, 67.60; H, 5.67; N, 9.85
Found: C, 67.29; H, 6.91; N, 10.18.

11. DL-N-Benzoyl-δ-o-aminophenylnorvaline

DL-N-Benzoyl-δ-o-aminophenylnorvaline amide (2g) was suspended in 20 ml dilute hydrochloric acid and warmed gently on a steam bath for 30 min. The contents were neutralised by dilute ammonia solution and the product was crystallised on cooling. This was filtered, washed with cold water and dried in air. The dried material melted at 154-55° and weighed 1.92g. The yield was almost quantitative. Recrystallisation from n-hexane-chloroform mixture (3:2) raised the melting point to 158-59°.

Anal for $C_{18}H_{20}O_3N_2$, Calcd: C, 69.21; H, 6.45; N, 8.97
Found: C, 69.51; H, 6.26; N, 9.29.

12. DL-N-Benzoylnorleucine

DL-N-Benzoylnorleucine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid, warmed on a steam bath for 2 hr to obtain a clear solution ans then left at room temperature for 8 hr. The crystalline material thus separated was filtered and washed with water to free the hydrochloric acid. On drying it weighed 1.5g (75%) and melted at 136-37° (lit[276] mp 136°). No depression in a mixed melting point determination with an authentic sample was observed.

13. DL-N-Benzoyl- δ; δ -dimethyl- δ -aminoisoleucine

DL-N-Benzoyl- δ; δ -dimethyl- δ -aminoisoleucine amide (2g) was suspended in dilute hydrochloric acid (20 ml) for 10 min. The acid was neutralised with ammonia solution and then was dried under reduced pressure. The residue was dissolved in 15 ml chloroform. To this 30 ml of benzene was added and the product crystallised on cooling melted at 166-67° and weighed 1.48g (74%).

Anal for $C_{15}H_{22}O_3N_2$, Calcd: C, 64.72; H, 7.97; N, 10.07
Found: C, 64.49; H, 6.86; N, 10.24.

14. DL-N-Benzoyl- δ; δ -dimethylisoleucine

DL-N-Benzoyl- δ; δ -dimethylisoleucine amide (2g) was heated with 40 ml of concentrated hydrochloric acid on a steam bath for 2 hr. After this period it was diluted with 30 ml of water and left at room temperature

overnight. The white crystalline product was filtered, washed with three 20-ml portions of water and dried in air. The benzoylamino acid thus obtained melted at 115-16° and weighed 1.1g. Mother liquor on concentration yielded another 0.35g of the product. Total yield was 1.45g (72.8%). Recrystallisation from n-hexane did not effect the melting point.

Anal for $C_{15}H_{21}O_3N$, Calcd: C, 68.41; H, 8.04; N, 5.32
Found: C, 68.49; H, 8.28; N, 5.69

15. DL-N-Benzoyl-β,γ-dimethylleucine

DL-N-Benzoyl-β,γ-dimethylleucine amide (2g) was suspended in 40 ml of concentrated hydrochloric acid. This was warmed on a steam bath for 5 hr and then left at room temperature for 10 hr. The contents were diluted with 30 ml of water and again left at room temperature overnight. The crystalline white product was filtered, washed with water and dried, mp 112-13°. Recrystallisation from 40% ethanol raised the melting point to116-17°. The yield, 1.94g (97%) was almost quantitative.

Anal for $C_{15}H_{21}O_3N$, Calcd: C, 68.41, H, 8.04; N, 5.32
Found: C, 68.75; H, 7.51; N, 5.73.

16. DL-N-Benzoyl-β-3-pyridylalanine

DL-N-Benzoyl-β-3-pyridylalanine amide (2g) was suspended in 20 ml of dilute hydrochloric acid (10%) and heated gently on a steam bath for 15 min. The solution was dried under reduced pressure and the residue so

secured was dissolved in 20 ml of water and then neutralised carefully with dilute ammonia solution. Crystallisation was effected by evaporating the contents to dryness, dissolving the residue in methanol and cooling the contents overnight. The crystalline product thus obtained weighed 1.42g (71%) and melted at 184-85° (lit[279] mp 186°).

Anal for $C_{15}H_{14}O_3N_2$; Calcd: C, 66.66; H, 5.22; N, 10.37
Found: C, 66.91; H, 5.37; N, 10.04.

17. DL-N-Benzoyl-β-piperonylalanine

DL-N-Benzoyl-β-piperonylalanine amide (2g) was suppended in 40 ml of concentrated hydrochloric acid. This was warmed on a steam bath for 20 min. The contents were diluted with 30 ml of water and left at room temperature overnight. The white crystalline product obtained in this manner was filtered, washed with three 20 ml portions of water and dried at 80° when it weighed 1.74g (74%) and melted at 166-67°. On recrystallisation from 20% ethanol, the benzoylamino acid melted at 171-72° (lit[214,277] mp 181-82° and 168-70°).

Anal for $C_{16}H_{15}O_5N$, Calcd: C, 65.17; H, 4.82; N, 4.47
Found: C, 64.88; H, 5.03; N, 4.74.

IV. PREPARATION OF AMINO ACIDS

1. DL-β-1-Naphthylalanine

DL-N-Benzoyl-β-1-naphthylalanine amide (2g) was added to a mixture of 20 ml of concentrated hydriodic acid (sp. gr. 1.7) and red phosphorus (1.5g) and refluxed for 2 hr. Then the mixture was cooled, filtered and the filtrate was evaporated under reduced pressure to dryness. The residue was taken up in 25 ml of water and evaporation to dryness was repeated. The residue thus obtained was suspended again in water and extracted several times with ether to remove benzoic acid and other impurities. The aqueous solution was heated on a steam bath to remove ether and then cautionly neutralised with dilute ammonia solution, heated on a steam bath and amino acid precipitated was filtered, washed with ice cold water and dried. It weighed 1.01g (75%) and melted at 237-38°. Recrystallisation from hot water raised the melting point to 244-45° dec (lit[103,284] mp 240°).

Anal for $C_{13}H_{13}O_2N$, Calcd: C, 72.55; H, 6.04; N, 6.51

Found: C, 72.82; H, 5.71; N, 6.84.

2. DL-β-o-Methoxyphenylalanine

DL-N-Benzoyl-β-o-methoxyphenylalanine amide (2g) was refluxed with 60 ml of concentrated hydrochloric acid (36%) for 13 hr and then left at room temperature overnight. Next morning benzoic acid separated was filtered and washed three times with 10 ml portions of ice cold water.

The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in 30 ml water and treated with silver oxide (2g). The contents were warmed on a steam bath for 1 hr with frequent shaking and filtered. The filtrate was treated with water-washed hydrogen shulphide gas to remove traces of silver, boiled on a free flame for few minutes and filtered. The clear filtrate was decolourised by heating with animal charcoal on a steam bath. This was concentrated to 15 ml, ethanol (20 ml) was then added and the solution was left for crystallisation at room temperature. The crystallised amino acid was filtered, washed twice with 10-ml portions of ethanol (95%), dried and weighed, 0.75g (56%), mp 238-39^{0}/^{dec} (lit^{280} mp 206^{0}).

Anal for $C_{10}H_{13}O_3N$, Calcd: C, 61.53; H, 6.71; N, 7.18
Found: C, 61.79; H, 6.39; N, 7.38.

3. DL-β-2;4-Dihydroxyphenylalanine

DL-N-Benzoyl-β-2;4-dihydroxyphenylalanine amide (2g) was added to a mixture of 20 ml of concentrated hydriodic acid and red phosphorus (1.6g) and the whole was refluxed for 2 hr. The mixture was cooled, unreacted phosphorus was removed by filtration and the filtrate was evaporated under reduced pressure to dryness. The residue was taken up in 25 ml of water and the solvent evaporated again. The residue was suspended in water and extracted several times with ether. The aqueous layer was heated on a steam bath and then treated with silver oxide (2g). This was warmed on a steam bath for 1 hr, filtered and the filtrate was treated with water-

washed hydrogen sulphide gas followed by boiling and filtration. The filtrate was then concentrated to 15 ml, 40 ml of ethanol (95%) was added and kept for crystallisation. The white, needle-shaped crystals of the amino acid thus separated were filtered, washed with ethanol and dried in an oven at 80°. It weighed 0.93g (71%) and melted at 223-24° with decomposition (lit[233,285] 257° dec. and 224°).

Anal for $C_9 H_{11} O_4 N$, Calcd: C, 54.82; H, 5.62; N, 7.10
Found: C, 54.92; H, 5.73; N, 7.29.

4. DL-β-3;4-Dihydroxyphenylalanine

DL-N-Benzoyl-β-3-methoxy-4-hydroxyphenylalanine amide (3g) was added to a mixture of concentrated hydriodic acid (20 ml), red phosphorus (2g) and glacial acetic acid (10 ml). This was refluxed in a stream of coal gas for 2 hr. The solution was filtered through asbestos and evaporated under reduced pressure to dryness. The residue was taken up in 25 ml of water and the solvent evaporated again. The residue was dissolved in 20 ml of water and extracted with three 25 ml of ether. Most of the pigment formed was decolourised by heating with animal charcoal, aqueous solution was evaporated under reduced pressure to a small bulk and then covered with a layer of n-hexane. This was neutralised carefully with concentrated ammonia solution, 20 ml of ethanol (95%) was then added containing a little sulphur dioxide gas. The amino acid was crystallised in vocuum on chilling, filtered,

dried when it melted at 288-89° dec (lit[212,79] mp 272° and 295°). The product thus obtained weighed 1.82g (90%). Mixed melting point with an authentic sample was not depressed.

Anal for $C_9 H_{11} O_4 N$, Calcd: C, 54.82; H, 5.62; N, 7.10

Found: C, 55.09; H, 5.41; N, 7.35.

5. DL-β-Amino-β-(o-benzenecarboxylic Acid) Alanine

DL-N-Benzoyl-β-amino-β-(o-benzenecarbonamido) alanine amide (2g) was refluxed with 25 ml of concentrated hydrochloric acid for 9 hr and then allowed to stand overnight at room temperature. Filtration of crystallised benzoic acid was effected over a Buchner funnal and this was washed with three successive portions (10 ml) of ice cold water. The combined filtrate and washings were evaporated to dryness. The residue was dissolved in 30 ml of water and treated with silver oxide (2g) on a steam bath. The precipitated silver chloride was filtered and the filtrate was saturated with hydrogen sulphide gas to precipitate traces of silver ions. This was filtered and the filtrate was concentrated to small bulk when the amino acid was emerged in crystalline form by the addition of 15 ml of ethanol. The yield of the product was 0.82g (60%), mp 244-45° (dec).

Anal for $C_{10} H_{12} O_4 N_2$, Calcd: C, 53.57; H, 5.39, N, 12.50

Found: C, 53.84, H, 5.10; N, 12.23

6. DL-β-p-Dimethylaminophenylalanine

DL-N-Benzoyl-β-p-dimethylaminophenylalanine amide (2g) was refluxed with 50 ml of dilute hydrochloric acid for 5 hr and then left at room temperature overnight. Benzoic acid thus separated was filtered, washed with three 10 ml portions of water and the solution was evaporated to dryness under reduced pressure. The amino acid salt so obtained was dissolved in 25 ml of water, neutralised with dilute ammonia solution, warmed on a steam bath and then 40 ml of ethanol was added. This solution was cool ed overnight and the crystalline amino acid separated was filtered washed with ethanol and dried in an oven at 80° when it weighed 0.95g (71-5%) and melted at 236-37° with decomposition (lit[278] mp 250 dec).

Anal for $C_{11}H_{16}O_2N_2$, Calcd: C, 63.44; H, 7.74; H, 13.45
Found: C, 63.67; H, 7.49; N, 13.51.

7. DL-Cyclopentylglycine

DL-N-Benzoylcyclopentylglycine amide (2g) was refluxed with concentrated hydrochloric acid for 18 hr and then left at room temperature overnight. Filtration of crystalline benzoic acid was effected over a Buchner funnel followed by washing with water (30 ml). The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was dissolved in 30 ml of water, treated with silver oxide (2g) and then warmed on a steam bath. The silver chloride formed in this

manner was filtered, the filtrate was saturated with hydrogen sulphide gas and filtered again. This was concentrated to 15 ml and crystallisation was effected by the addition of ethyl acetate-ethanol mixture (1:4). The yield of dried amino acid obtained was 0.73g (63%), mp 298-99° (dec) (lit[281] mp 284-86 and 250[9] dec).

Anal for $C_7 H_{13} O_2 N$, Calcd: C, 58.72; H, 9.15; N, 9.78
Found: C, 59.01, H, 9.23; N, 10.10.

8. DL-Cyclohexylglycine

DL-N-Benzoylcyclohexylglycine amide (2g) was refluxed for 2 hr with a mixture of concentrated hydriodic acid (20 ml) and red phosphorus (1.5g). Unreacted phosphorus was removed by filtration. The filtrate was evaporated to dryness under reduced pressure, 20 ml of water was added and the evaporation to dryness was repeated. The residue obtained in this manner was suspended in water (25 ml) and extracted several times with ether. The aqueous solution was neutralised with ammonia solution and heated on a steam bath for 15 minutes. The precipitated amino acid was filtered, washed with three 10 ml portions of water and then with 15 ml of acetone. On drying the amino acid melted at 269-80° (dec) and weighed (0.76g (64%). Recrystallisation from glacial acetic acid raised the melting point to 296-97° (dec) (lit[137] mp 225° dec).

Anal for $C_8 H_{15} O_2 N$, Calcd: C, 61.12; H, 9.62; N, 8.91
Found: C, 60.86; H, 9.47; N, 9.19

9. DL-o-Tyrosine

DL-N-Benzoyl-o-tyrosine amide (2g) was refluxed with a mixture of concentrated hydriodic acid (20 ml) and red phosphorus (2g) for 2 hr. The solution was filtered through asbestos and dried under reduced pressure. The residue was taken up in 10 ml of water, the solvent was re-evaporated and the residue was again dissolved in water (25 ml). This was extracted thrice with 25 ml portions of ether and treated with silver oxide (2g) on a steam bath for 1 hr. precipitated silver iodide was filtered and the solution was saturated with hydrogen sulphide gas to remove the traces of silver ions. This was filtered and the filtrate was concentrated to 15 ml, ethanol (30 ml) was added and this was cooled in a refrigerator overnight. The amino acid thus separated was filtered, washed with ethanol and dried when it melted at 218-19° (lit[275,280] mp 250° and 232°) and weighed 1.12g (89%).

Anal for $C_9 H_{11} O_3 N$, Calcd: C, 59.68, H, 6.12; N, 7.73
Found: C, 59.86; H, 5.83; N, 8.03.

10. DL-β-m-Aminophenylalanine

DL-N-Benzoyl-β-m-aminophenylalanine amide (2g) was refluxed with dilute hydrochloric acid (30 ml) for 2 hr and then left at room temperature overnight. Benzoic acid thus separated was removed through filtration and the filtrate was dried under reduced pressure. The residue was dissolved in 25 ml of water and then neutralised with dilute ammonia solution. This was concentrated to 15 ml, ethanol (40 ml)

was added and the solution was left for crystallisation. The amino acid thus obtained was filtered, washed with ethanol and dried in an oven at 80° when at weighed 0.96g (79%) and melted at 304-5° with decomposition (lit[283] mp 248-52).

Anal for $C_9 H_{12} O_2 N_2$, Calcd: C, 59.98; H, 6.71; N, 15.55
Found: C, 59.85; H, 6.79; N, 15.86.

11. DL-δ-o-Aminophenylnorvaline

DL-N-Benzoyl-δ-o-aminophenylnorvaline amide (2g) was heated under reflux with dilute hydrochloric acid (20 ml) for 2 hr and then left at room temperature overnight. Filtration of crystallised benzoic acid was effected over a Buchner funnel and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 25 ml of water, treated with silver oxide (2g) on a steam bath for 1 hr and filtered. The filtrate was saturated with hydrogen sulphide gas, boiled for 5 min and filtered. The filtrate was evaporated to dryness and the residue obtained in this way was crystallised from alcohol-ethyl acetate mixture (3:2) when 0.98g (74%) of the amino acid was obtained, mp 160-61°.

Anal for $C_{11} H_{16} O_2 N_2$, Calcd: C, 63.44; H, 7.74; N, 13.45
Found: C, 63.75; H, 7.43; N, 13.71.

12. DL-Norleucine

DL-N-Benzoylnorleucine amide (2.5g) was refluxed with a mixture of concentrated hydriodic acid (20 ml) and red phosphorus (2g) for 1.5 hr. The mixture was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was taken up in 25 ml of water and the solvent re-evaporated. The residue was suspended again in water (25 ml), extracted several times with ether and the solution was heated for few minutes on a steam bath. This was neutralised with ammonia solution, and then concentrated to 15 ml. Ethanol (20 ml) was added when the amino acid crystallised on cooling which was filtered and dried. It weighed 1.32g (95%) and melted at 296-97° dec (lit[282] mp. 291-5°). Mixed melting point with an authentic sample was not depressed.

13. DL- δ;δ -Dimethyl-δ-aminoisoleucine

DL-N-Benzoyl- δ;δ-dimethyl-δ-aminoisoleucine amide (2g) was heated under reflux with dilute hydrochloric acid (40 ml) for 1 hr and then left at room temperature overnight. Benzoic acid which separated was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 25 ml of water and treated with silver oxide (3g) on a steam bath for 1 hr. This was filtered and the filtrate was saturated with hydrogen sulphide gas. Silver sulphide thus precipitated was filtered and then concentrated to 10 ml. To this ethanol (20 ml) was added and left for crystallisation. The amino acid

obtained in this manner melted at 264-65° (dec) and weighed 0.79g (62%).

Anal for $C_8 H_{18} O_2 N_2$, Calcd: C, 55.14; H, 10.41; N, 16.08

Found: C, 55.42; H, 10.15; N, 16.29.

14. DL- $\delta;\delta$ -Dimethylisoleucine

DL-N-Benzoyl- $\delta;\delta$ -dimethylisoleucine amide (3g) was refluxed with concentrated hydrochloric acid (50 ml) for 9 hr and then left at roomtemperature overnight. Filtration of crystalline benzoic acid was effected over a Buchner funnel and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 25 ml of water and treated with silver oxide (2.5g) on a steam bath for 1 hr. This was filtered, the filtrate was saturated with hydrogen sulphide gas and filtered again. The filtrate was concentrated to 10 ml, ethanol (30 ml) was then added and the amino acid obtained on cooling was filtered. On drying it melted at 272-74° (dec) and weighed 1 g. Mother liquor on concentration gave another 0.45g of the product. The total yield was 1.45g (78%).

Anal for $C_8 H_{17} O_2 N$, Calcd: C, 60.34; H, 10.76; N, 8.80

Found: C, 60.04; H, 10.89; N, 9.12

15. DL- $\beta;\gamma$ -Dimethylleucine

DL-N-Benzoyl- $\beta;\gamma$ -dimethylleucine amide (2.5g) was refluxed with concentrated hydrochloric acid (50 ml) for 16 hr and then left at

room temperature overnight. After separating crystalline benzoic acid by filtration, the filtrate was dried under reduced pressure and the residue was dissolved in 20 ml of water. This was treated with silver oxide (2g) on a steam bath for 1 hr and filtered. The filtrate was saturated with hydrogen sulphide gas, heated for few minutes and filtered again. The filtrate was concentrated to 15 ml, ethanol (30 ml) was then added when the amino acid crystallised on cooling. This was filtered and dried when 1.18g (82%) of the amino acid was obtained, mp 278-79° (dec).

Anal for $C_8 H_{17} O_2 N$, Calcd: C, 60.34; H, 10.76; N, 8.80
Found: C, 60.48; H, 10.78; N, 8.61.

16. DL-β-3-Pyridylalanine

DL-N-Benzoyl-β-3-pyridylalanine amide (2.5g) was heated under reflux with dilute hydrochloric acid (25 ml 10%) for 1.5 hr and then left at room temperature overnight. Filtration of crystallised benzoic acid was effected over a Buchner funnel and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 25 ml of water, treated with silver oxide (4 g) on a steam bath for 1 hr and filtered. The filtrate was saturated with hydrogen sulphide gas, boiled for 5 min and then refiltered. The filtrate thus obtained was concentrated to 15 ml, ethanol (40 ml) was added and this was cooled for crystallisation. The amino acid separated was filtered, washed with ethanol and dried when it weighed

1g. On concentrating the mother liquor a second crop of crystals weighing 0.5g was obtained. The total yield was 1.5g (nearly quantitative), mp 279-80° dec (lit[257] mp 266.5)

Anal for $C_8 H_{10} O_2 N_2$: Calcd: C, 57.82; H, 6.07; N, 16.86

Found: C, 57.91; H, 5.86; N, 17.12.

17. DL-β-Piperonylalanine

DL-N-Benzoyl-β-piperonylalanine amide (2g) was refluxed with barium hydroxide solution (40 ml, 15%) for 12 hr. At the end of this period it was diluted with 50 ml of water and calculated amount of dilute sulphuric acid was added with thorough shaking to neutralise the alkali. The precipitated barium sulphate was filtered and washed with three successive 20 ml portions of water. The combined filtrate and washings were evaporated to dryness under reduced pressure and the residue so obtained was crystallised from aqueous ethanol (50%). The amino acid obtained in this manner melted at 230-31° (dec) and weighed 1.03g (73-5%) (lit[211] mp 262-64°).

Anal for $C_{10} H_{11} O_4 N$, Calcd: C, 57.41; H, 5.30; N, 6.70

Found: C, 57.59; H, 5.51; N, 6.63.

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